

(FILE 'HOME' ENTERED AT 15:08:07 ON 21 FEB 2002)

FILE 'MEDLINE, AGRICOLA, CAPLUS, BIOSIS, EMBASE, WPIDS' ENTERED AT  
15:08:16 ON 21 FEB 2002

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L1      65212 S MONOOXYGENASE
L2      111 S L1 AND (TYPE (W) (3 OR III))
L3      16 S L2 AND LIVER
L4      6 S L3 AND HUMAN
L5      3 DUP REM L4 (3 DUPLICATES REMOVED)
L6      561 S FMO3 OR FMOS OR FMO-3 OR FMO-III
L7      561 S FMO3 OR FMOS OR FMO-3 OR FMO-III OR FMOIII
L8      243 S L7 AND HUMAN AND LIVER
L9      243 S L7 AND (HUMAN OR SAPIENS) AND LIVER
L10     62 S L9 AND (DNA OR POLYNUCLEOTIDE OR NUCLEIC OR NUCLEOTIDE)
L11     124 S L9 AND (DNA OR POLYNUCLEOTIDE OR NUCLEIC OR NUCLEOTIDE OR RN
L12     125 S L9 AND (DNA OR POLYNUCLEOTIDE OR NUCLEIC OR NUCLEOTIDE OR RN
L13     42 DUP REM L12 (83 DUPLICATES REMOVED)
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FILE 'CAPLUS' ENTERED AT 15:17:04 ON 21 FEB 2002

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      E CASHMAN L/AU 25
      E CASHMAN J/AU 25
L14     48 S (E3 OR E6 OR E13 OR E15 OR E16) AND (MONOOXYGENASE AND LIVER)
L15     20 S (E3 OR E6 OR E13 OR E15 OR E16) AND (MONOOXYGENASE AND LIVER
      E LOMRI N/AU 25
L16     5 S (E3 OR E4 OR E5 OR E6 OR E7) AND (MONOOXYGENASE AND LIVER AND
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CONTINUE? Y/(N):y

L13 ANSWER 1 OF 42 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 2002045707 EMBASE

TITLE: Cloning, sequencing, and tissue-dependent expression of flavin-containing monooxygenase (FMO) 1 and FMO3 in the dog.

AUTHOR: Lattard V.; Longin-Sauvageon C.; Lachuer J.; Delatour P.; Benoit E.

CORPORATE SOURCE: Dr. E. Benoit, Unite de Toxicologie, UMR INRA et DGER, Ecole Nationale Veterinaire de Lyon, BP 83, 69280 Marcy l'etoile, France. e.benoit@vet-lyon.fr

SOURCE: Drug Metabolism and Disposition, (2002) 30/2 (119-128).

Refs: 33

ISSN: 0090-9556 CODEN: DMDSAI

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 029 Clinical Biochemistry

LANGUAGE: English

SUMMARY LANGUAGE: English

AB The expression of flavin-containing monooxygenases (FMOs) in dog liver microsomes was suggested by a high methimazole S-oxidase activity. When the reaction was catalyzed by dog liver microsomes, apparent V(max) and K(m) values were 6.3 nmol/min/mg and 14 .mu.M, respectively. This reaction was highly inhibited (73%) in the presence of imipramine, but it was also weakly affected by trimethylamine, suggesting the involvement of different isoforms. The sequences of dog FMO1 and FMO3 were obtained by reverse transcription-polymerase chain reaction and 5'/3' terminal extension. The cDNAs of dog FMO1 and dog FMO3 encode proteins of 532 amino acids, which contain the NADPH- and FAD-binding sites. The dog FMO1 amino acid sequence is 88, 86, and 89% identical to sequences of human, rabbit, and pig FMO1, respectively. The dog FMO3 amino acid sequence is 83, 84, and 82% identical to sequences of human, rabbit, and rat FMO3, respectively. Dog FMO1 and dog FMO3 exhibited only 56% identities. The FMO1 and FMO3 recombinant proteins and the FMO1 and FMO3 microsomal proteins migrated with the same mobility (56 kDa), as determined in SDS-polyacrylamide gel electrophoresis and immunoblotting. By Western blotting, dog FMO1 and dog FMO3 were detected in microsomes from liver and lung but not in kidney microsomes. By Northern blotting, the probe for FMO1 specifically hybridized a 2.6-kilobase (kb) transcript in liver and lung samples only. The probe for FMO3 hybridized two transcripts of approximately 3 and 4.2 kb in the liver and lung samples.

L13 ANSWER 2 OF 42 MEDLINE

ACCESSION NUMBER: 2001677283 MEDLINE

DOCUMENT NUMBER: 21580262 PubMed ID: 11723251

TITLE: Regulation of flavin-containing monooxygenase 1 expression by ying yang 1 and hepatic nuclear factors 1 and 4.

AUTHOR: Luo Z; Hines R N

CORPORATE SOURCE: Departments of Pediatrics and Pharmacology and Toxicology, Birth Defects Research Center, Medical College of Wisconsin, Milwaukee, Wisconsin 53226-4801, USA.

CONTRACT NUMBER: CA53106 (NCI)

SOURCE: MOLECULAR PHARMACOLOGY, (2001 Dec) 60 (6) 1421-30.

Journal code: 0035623. ISSN: 0026-895X.

PUB. COUNTRY: United States

Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

OTHER SOURCE: GENBANK-AF355464

ENTRY MONTH: 200112

ENTRY DATE: Entered STN: 20011128

Last Updated on STN: 20020123

Entered Medline: 20011221

AB The flavin-containing monooxygenases (FMOs) are important for the oxidation of a variety of environmental toxicants, natural products, and therapeutics. Consisting of six family members (FMO1-5), these enzymes exhibit distinct but broad and overlapping substrate specificity and are expressed in a highly tissue- and species-selective manner. Corresponding to previously identified regulatory domains, a YY1 binding site was identified at the major rabbit FMO1 promoter, position -8 to -2, two overlapping HNF1alpha sites, position -132 to -105, and two HNF4alpha sites, position -467 to -454 and -195 to -182. Cotransfection studies with HNF1alpha and HNF4alpha expression vectors demonstrated a major role for each of these factors in enhancing FMO1 promoter activity. In contrast, YY1 was shown by site-directed mutagenesis to be dispensable for basal

promoter activity but suppressed the ability of the upstream domains to enhance transcription. Finally, comparisons between rabbit and human FM01 demonstrated conservation of each of these regulatory elements. With the exception of the most distal HNF4alpha site, each of the orthologous human sequences also was able to compete with rabbit FM01 cis-elements for specific protein binding. These data are consistent with these same elements being important for regulating human FM01 developmental- and tissue-specific expression.

L13 ANSWER 3 OF 42 MEDLINE DUPLICATE 1  
 ACCESSION NUMBER: 2001679458 IN-PROCESS  
 DOCUMENT NUMBER: 21582395 PubMed ID: 11725960  
 TITLE: Identification of human drug-metabolizing enzymes involved in the metabolism of SNI-2011.  
 AUTHOR: Washio T; Arisawa H; Kohsaka K; Yasuda H  
 CORPORATE SOURCE: Research Institute of Life Science, Snow Brand Milk Products Co, Ltd, Shimotsuga-gun, Tochigi, Japan.. washio-t-e@pop16.odn.ne.jp  
 SOURCE: BIOLOGICAL AND PHARMACEUTICAL BULLETIN, (2001 Nov) 24 (11) 1263-6.  
 Journal code: 9311984. ISSN: 0918-6158.  
 PUB. COUNTRY: Japan  
 Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: IN-PROCESS; NONINDEXED; Priority Journals  
 ENTRY DATE: Entered STN: 20011203  
 Last Updated on STN: 20020123

AB In vitro studies were conducted to identify human drug-metabolizing enzymes involved in the metabolism of SNI-2011 ((+/-)-cis-2-methylspiro [1,3-oxathiolane-5,3'-quinuclidine] monohydrochloride hemihydrate, cevimeline hydrochloride hydrate). When 14C-SNI-2011 was incubated with human liver microsomes, SNI-2011 trans-sulfoxide and cis-sulfoxide were detected as major metabolites. These oxidations required NADPH, and were markedly inhibited by SKF-525A, indicating that cytochrome P450 (CYP) was involved. In a chemical inhibition study, metabolism of SNI-2011 in liver microsomes was inhibited (35-65%) by CYP3A4 inhibitors (ketoconazole and troleandomycin) and CYP2D6 inhibitors (quinidine and chlorpromazine). Furthermore, using microsomes containing cDNA-expressed CYPs, it was found that high rates of sulfoxidation activities were observed with CYP2D6 and CYP3A4. On the other hand, when 14C-SNI-2011 was incubated with human kidney microsomes, SNI-2011 N-oxide was identified as a major metabolite. This N-oxidation required NADPH, and was completely inhibited by thiourea, indicating that flavin-containing monooxygenase (FMO) was involved. In addition, microsomes containing cDNA-expressed FM01, a major isoform in human kidney, mainly catalyzed N-oxidation of SNI-2011, but microsomes containing FM03, a major isoform in adult human liver, did not. These results suggest that SNI-2011 is mainly catalyzed to sulfoxides and N-oxide by CYP2D6/3A4 in liver and FMO1 in kidney, respectively.

L13 ANSWER 4 OF 42 MEDLINE DUPLICATE 2  
 ACCESSION NUMBER: 2001501077 MEDLINE  
 DOCUMENT NUMBER: 21435636 PubMed ID: 11551524  
 TITLE: Quantification and cellular localization of expression in human skin of genes encoding flavin-containing monooxygenases and cytochromes P450.  
 AUTHOR: Janmohamed A; Dolphin C T; Phillips I R; Shephard E A  
 CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, University College London, London, UK.  
 SOURCE: BIOCHEMICAL PHARMACOLOGY, (2001 Sep 15) 62 (6) 777-86.  
 Journal code: 924; 0101032. ISSN: 0006-2952.  
 PUB. COUNTRY: England; United Kingdom  
 Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 200109  
 ENTRY DATE: Entered STN: 20010912  
 Last Updated on STN: 20010924  
 Entered Medline: 20010920

AB The expression, in adult human skin, of genes encoding flavin-containing monooxygenases (FMOs) 1, 3, 4, and 5 and cytochromes P450 (CYPs) 2A6, 2B6, and 3A4 was determined by RNase protection. Each FMO and CYP exhibits inter-individual variation in expression in this organ. Of the individuals analysed, all contained CYP2B6 mRNA in their skin, 90% contained FMO5 mRNA and about half contained mRNAs encoding FMOs 1, 3, and 4, and CYPs 2A6 and 3A4. The amount of each of the FMO and CYP mRNAs in skin is much

lower than in the organ in which it is most highly expressed, namely the kidney (for FMO1) and the liver (for the others). In contrast to the latter organs, in the skin FMO mRNAs are present in amounts similar to, or greater than, CYP mRNAs. Only the mRNA encoding CYP2B6 decreased in abundance in skin with increasing age of the individual. All of the mRNAs were substantially less abundant in cultures of keratinocytes than in samples of skin from which the cells were derived. In contrast, an immortalized human keratinocyte cell line, HaCaT, expressed FMO3, FMO5, and CYP2B6 mRNAs in amounts that fall within the range detected in the whole skin samples analysed. FMO1, CYP2A6, and CYP3A4 mRNAs were not detected in HaCaT cells, whereas FMO4 expression was markedly increased in this cell line compared to whole skin. In situ hybridization showed that the expression of each of the FMOs and CYPs analysed was localized to the epidermis, sebaceous glands and hair follicles.

L13 ANSWER 5 OF 42 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 2001:306993 BIOSIS

DOCUMENT NUMBER: PREV200100306993

TITLE: Over production of nitric oxide and peroxynitrite in patients with liver cirrhosis and cancer suppresses flavin-containing monooxygenase (FMO) activity and causes trimethylaminuria (TMAU).

AUTHOR(S): Yi, H. G.; Park, C. S.; Kang, J. S.; Kang, J. H.; Chung, W. G.; Pie, J. E.; Ryu, S. D.; Choi, W.; Cha, Y. N.

SOURCE: FASEB Journal, (March 7, 2001) Vol. 15, No. 4, pp. A576. print.

Meeting Info.: Annual Meeting of the Federation of American Societies for Experimental Biology on Experimental Biology 2001 Orlando, Florida, USA March 31-April 04, 2001  
ISSN: 0892-6638.

DOCUMENT TYPE: Conference

LANGUAGE: English

SUMMARY LANGUAGE: English

AB In 5 patients with liver cirrhosis and cancer, trimethylaminuria (TMAU) also known as Fish Odor Syndrome, a congenital metabolic disorder generally caused by gene defect in flavin-containing monooxygenase, is observed. In human, major part of FMO3 is expressed in the liver and catalyzes the N-oxidation of volatile trimethylamine (TMA) derived from choline diets to non-volatile TMA N-oxide excreted in urine. Thus, when the hepatic FMO3 activity is suppressed by hepatitis, cirrhosis and cancer, TMAU can be acquired. Prior to surgery, the in vivo FMO activities determined by urinary excretion of TMA N-oxide derived from normal diet were found to be low. Furthermore, the in vitro FMO activities (N-oxidation of ranitidine) determined with liver microsomes of patients similarly affected were found to be very low. Conversely, the plasma concentration of NO metabolites was increased by 3-6 fold of healthy volunteers. In the cirrhotic liver tissues obtained from patients after surgery, the contents of FMO3 mRNA and protein were very low and in the cancerous tissues, they were almost absent. In the cirrhotic and cancerous liver tissues, however, the expected over-expression of iNOS could not be clearly detected (immunohistochemistry and Western-blot). FMO3 present in the human liver microsomes obtained from these surgical tissues was found to be nitrated in Western-blot. This in vivo FMO3 nitration result was confirmed in vitro by treating human liver microsomes obtained from normal liver with SIN-1, a donor of peroxynitrite (ONOO-). Combined, these results suggest that the over-produced NO and ONOO under a LPS-induced septic condition (Park et al., 1999) or a liver cirrhosis and cancer (present study) may cause TMAU both by suppression of FMO gene expression and by nitration of expressed FMO.

L13 ANSWER 6 OF 42 MEDLINE . DUPLICATE 3

ACCESSION NUMBER: 2001165255 MEDLINE

DOCUMENT NUMBER: 21163847 PubMed ID: 11266081

TITLE: A novel deletion in the flavin-containing monooxygenase gene (FMO3) in a Greek patient with trimethylaminuria.

AUTHOR: Forrest S M; Knight M; Akerman B R; Cashman J R; Treacy E P

CORPORATE SOURCE: Murdoch Children's Research Institute, Royal Children's Hospital, Parkville, Victoria, Australia..  
forrest@cryptic.rch.unimelb.edu.au

CONTRACT NUMBER: GM36426 (NIGMS)

SOURCE: PHARMACOGENETICS, (2001 Mar) 11 (2) 169-74.  
Journal code: BRT; 9211735. ISSN: 0960-314X.

PUB. COUNTRY: England: United Kingdom

## 09/583,310 Search Strategy/Results

Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200106  
ENTRY DATE: Entered STN: 20010618  
Last Updated on STN: 20010618  
Entered Medline: 20010614

AB Mutations of the flavin-containing monooxygenase type 3 gene (FMO3) that encode the major functional form present in adult human liver, have been shown to cause trimethylaminuria. We now report a novel homozygous deletion of exons 1 and 2 in an Australian of Greek ancestry with TMAuria, the first report of a deletion causative of trimethylaminuria. The deletion occurs 328 bp upstream from exon 1. The 3'-end of the deletion occurs in intron 2, 10013 base pairs downstream from the end of exon 2. The deletion is 12226 bp long. For the proband homozygous for the human FMO3 gene deletion, it is predicted that in addition to loss of monooxygenase function for human FMO3 substrates, such as TMA and other amines, the proband will exhibit decreased tolerance of biogenic amines, both medicinal and those found in foods.

L13 ANSWER 7 OF 42 MEDLINE DUPLICATE 4  
ACCESSION NUMBER: 2001351709 MEDLINE  
DOCUMENT NUMBER: 21308417 PubMed ID: 11414682  
TITLE: Cloning, sequencing, tissue distribution, and heterologous expression of rat flavin-containing monooxygenase 3.  
AUTHOR: Lattard V; Buronfosse T; Lachuer J; Longin-Sauvageon C; Moulin C; Benoit E  
CORPORATE SOURCE: Unite de Toxicologie et de Metabolisme Comparees des Xenobiotiques, UMR INRA et DGER, Ecole Nationale Veterinaire de Lyon, 69280 Marcy l'etoile, France.  
SOURCE: ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, (2001 Jul 1) 391 (1) 30-40.  
JOURNAL CODE: 6SK; 0372430. ISSN: 0003-9861.  
PUB. COUNTRY: United States  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
OTHER SOURCE: GENBANK-AF286595  
ENTRY MONTH: 200107  
ENTRY DATE: Entered STN: 20010723  
Last Updated on STN: 20010723  
Entered Medline: 20010719

AB The sequence of rat FMO3 was obtained by RT-PCR and 5'/3' terminal extension. Complete cDNA was amplified, cloned, and sequenced. The cDNA encodes a protein of 531 amino acids which contains the NADPH- and FAD-binding sites and a hydrophobic carboxyl terminus characteristic of FMOs. This sequence is 81, 81, and 91% identical to sequences of human, rabbit, and mouse FMO3, respectively, and 60% identical to rat FMO1. Rat FMO3 was expressed in Escherichia coli. The recombinant protein and the native protein purified from rat liver microsomes migrated with the same mobility (56 kDa) as determined in sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting. Recombinant rat FMO3 showed activities of methimazole S-oxidation, and NADPH oxidation associated with the N- or S-oxidation of trimethylamine and thioacetamide, in good concordance with those reported for human FMO3. When probed with rat FMO3 cDNA (bases 201 to 768), a strong signal corresponding to the 2.3-kb FMO3 transcript was detected in RNA samples from rat liver and kidney while a weak signal was observed with lung RNA samples. In contrast, the probe did not hybridize with any RNA from brain, adipose tissue, or muscle. Copyright 2001 Academic Press.

L13 ANSWER 8 OF 42 MEDLINE DUPLICATE 5  
ACCESSION NUMBER: 2001030942 MEDLINE  
DOCUMENT NUMBER: 20493209 PubMed ID: 11038163  
TITLE: In vitro evaluation of the disposition of A novel cysteine protease inhibitor.  
AUTHOR: Jacobsen W; Christians U; Benet L Z  
CORPORATE SOURCE: Department of Biopharmaceutical Sciences, School of Pharmacy, University of California, San Francisco, California 94143-0446, USA.  
CONTRACT NUMBER: CA72006 (NCI)  
SOURCE: DRUG METABOLISM AND DISPOSITION, (2000 Nov) 28 (11) 1343-51.  
JOURNAL CODE: EBR. ISSN: 0090-9556.  
PUB. COUNTRY: United States

(EVALUATION STUDIES)  
 Journal; Article; (JOURNAL ARTICLE)  
 English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 200011  
 ENTRY DATE: Entered STN: 20010322  
 Last Updated on STN: 20010322  
 Entered Medline: 20001120

AB K11777 (N-methyl-piperazine-Phe-homoPhe-vinylsulfone-phenyl) is a potent, irreversible cysteine protease inhibitor. Its therapeutic targets are cruzain, a cysteine protease of the protozoan parasite *Trypanosoma cruzi*, and cathepsins B and L, which are associated with cancer progression. We evaluated the metabolism of K11777 by human liver microsomes, isolated cytochrome P450 (CYP) enzymes, and flavin-containing monooxygenase 3 (FMO3) in vitro. K11777 was metabolized by human liver microsomes to three major metabolites: N-oxide K11777 (apparent  $K(m) = 14.0 \pm 4.5$  microM and apparent  $V(max) = 3460 \pm 3190$  pmol. mg<sup>-1</sup>. min<sup>-1</sup>),  $n = 4$ ), beta-hydroxy-homoPhe K11777 ( $K(m) = 16.8 \pm 3.5$  microM and  $V(max) = 1260 \pm 1090$  pmol. mg<sup>-1</sup>. min<sup>-1</sup>),  $n = 4$ ), and N-desmethyl K11777 ( $K(m) = 18.3 \pm 7.0$  microM and  $V(max) = 2070 \pm 1830$  pmol. mg<sup>-1</sup>. min<sup>-1</sup>),  $n = 4$ ). All three K11777 metabolites were formed by isolated CYP3A and their formation by human liver microsomes was inhibited by the CYP3A inhibitor cyclosporine (50 microM, 54-62% inhibition) and antibodies against human CYP3A4/5 (100 microg of antibodies/100 microg microsomal protein, 55-68% inhibition). CYP2D6 metabolized K11777 to its N-desmethyl metabolite with an apparent  $K(m)$  ( $9.2 \pm 1.4$  microM) lower than for CYP3A4 ( $25.0 \pm 4.0$  microM) and human liver microsomes. The apparent  $K(m)$  for N-oxide K11777 formation by cDNA-expressed FMO3 was  $109 \pm 11$  microM. Based on the intrinsic formation clearances and the results of inhibition experiments (CYP2D6, 50 microM bufuralol; FMO3 mediated, 100 mM methionine) using human liver microsomes, it was estimated that CYP3A contributes to >80% of K11777 metabolite formation. K11777 was a potent ( $IC(50) = 0.06$  microM) and efficacious (maximum inhibition 85%) NADPH-dependent inhibitor of human CYP3A4 mediated 6'beta-hydroxy lovastatin formation, suggesting that K11777 is not only a substrate but also a mechanism-based inhibitor of CYP3A4.

L13 ANSWER 9 OF 42 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 6  
 ACCESSION NUMBER: 2000:614884 CAPLUS  
 DOCUMENT NUMBER: 133:290618  
 TITLE: Isoform specificity of N-deacetyl ketoconazole by human and rabbit flavin-containing monooxygenases  
 AUTHOR(S): Rodriguez, Rosita J.; Miranda, Cristobal L.  
 CORPORATE SOURCE: Department of Pharmaceutical Sciences, Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR, 97331-3507, USA  
 SOURCE: Drug Metab. Dispos. (2000), 28(9), 1083-1086  
 CODEN: DMSAI; ISSN: 0090-9556  
 PUBLISHER: American Society for Pharmacology and Experimental Therapeutics  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English

AB N-Deacetyl ketoconazole (DAK) is the major metabolite of orally administered ketoconazole. This major metabolite has been demonstrated to be further metabolized predominately by the flavin-contg. monooxygenases (FMOs) to the secondary hydroxyl-amine, N-deacetyl-N-hydroxyketoconazole (N-hydroxy-DAK) by adult and postnatal rat hepatic microsomes. Our current investigation evaluated the FMO isoform specificity of DAK in a pyrophosphate buffer (pH 8.8) contg. the glucose 6-phosphate NADPH-generating system. CDNA-expressed human FMOs (FMO1, FMO3, and FMO5) and cDNA-expressed rabbit FMOs (FMO1, FMO2, FMO3, and FMO5) were used to assess the metab. of DAK to its subsequent FMO-mediated metabolites by HPLC anal. Human and rabbit cDNA-expressed FMO3 resulted in extensive metab. of DAK in 1 h (71.2 and 64.5%, resp.) to N-hydroxy-DAK (48.2 and 47.7%, resp.) and two other metabolites, metabolite 1 (11.7 and 7.8%, resp.) and metabolite 3 (10.5 and 10.0%, resp.). Previous studies suggest that metabolite 1 is the nitron formed after successive FMO-mediated metab. of N-hydroxy-DAK. Moreover, these studies display similar metabolic profiles seen with adult and postnatal rat hepatic microsomes. The human and rabbit FMO1 metabolized DAK pre-dominately to the N-hydroxy-DAK in 1 h (36.2 and 25.3%, resp.) with minimal metab. to the other metabolites (<5%). Rabbit FMO2 metabolized DAK to N-hydroxy-DAK (15.9%) and metabolite 1 (6.6%). Last, DAK did not appear to be a substrate for

09/583,310 Search Strategy/Results

human or rabbit FMO5. Heat inactivation of cDNA  
-expressed FMOs abolished DAK metabolite formation. These  
results suggest that DAK is a substrate for human and rabbit  
FMO1 and FMO3, rabbit FMO2, but not human or rabbit  
FMO5.

REFERENCE COUNT: 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS  
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 10 OF 42 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 2001017163 EMBASE

TITLE: Compound heterozygosity for missense mutations in the  
flavin-containing monooxygenase 3 (FMO3) gene in patients  
with fish-odour syndrome.

AUTHOR: Dolphin C.T.; Jannmohamed A.; Smith R.L.; Shephard E.A.;  
Phillips I.R.

CORPORATE SOURCE: I.R. Phillips, Molecular and Cellular Biology, Division of  
Biomedical Sciences, Queen Mary and Westfield College, Mile  
End Road, London E1 4NS, United Kingdom.  
ir.phillips@qmw.ac.uk

SOURCE: Pharmacogenetics, (2000) 10/9 (799-807).

Refs: 29

ISSN: 0960-314X CODEN: PHMCEE

COUNTRY: United Kingdom

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 006 Internal Medicine  
022 Human Genetics  
029 Clinical Biochemistry

LANGUAGE: English

SUMMARY LANGUAGE: English

AB Fish-odour syndrome is a highly unpleasant disorder of hepatic  
trimethylamine (TMA) metabolism characterized by a body odour reminiscent  
of rotting fish, due to excessive excretion of the malodorous free amine.  
Although fish-odour syndrome may exhibit as sequelae with other conditions  
(e.g. liver dysfunction), many patients exhibit an inherited,  
more persistent form of the disease. Ordinarily, dietary-derived TMA is  
oxidized to the non-odorous N-oxide by hepatic flavin-containing  
monooxygenase 3 (FMO3). Our previous demonstration that a  
mutation, P153L (C to T), in the FMO3 gene segregated with the  
disorder and inactivated the enzyme confirmed that defects in FMO3  
underlie the inherited form of fish-odour syndrome. We have investigated  
the genetic basis of the disorder in two further affected pedigrees and  
report that the three propoiti are all compound heterozygotes for  
causative mutations of FMO3. Two of these individuals possess  
the P153L (C to T) mutation and a novel mutation, N61S (A to G). The third  
is heterozygous for novel. M4341 (G to A), and previously reported. R492W  
(C to T), mutations. Functional characterization of the S61, 1434 and W492  
variants, via baculovirus-mediated expression in insect cells, confirmed  
that all three mutations either abolished, or severely attenuated, the  
capacity of the enzyme to catalyse TMA N-oxidation. Although 1434 and W492  
were also incapable of catalysing the S-oxidation of methimazole, S61 was  
fully active with this sulphur-containing substrate. Since an asparagine  
is conserved at the equivalent position to N61 of FMO3 in  
mammalian, yeast and Caenorhabditis elegans FMOs, the  
characterization of the naturally occurring N61S (A to G) mutation may  
have identified this asparagine as playing a critical role specifically in  
FMO-catalysed N-oxidation. .COPYRG. 2000 Lippincott Williams & Wilkins.

L13 ANSWER 11 OF 42 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 2001:114393 BIOSIS

DOCUMENT NUMBER: PREV200100114393

TITLE: Sequence variation in the human flavin-containing  
monooxygenase 3 gene (FMO3): Identification by  
denaturing-high performance liquid chromatography (DHPLC).

AUTHOR(S): Charon, C. M. (1); Dolphin, C. T. (1)

CORPORATE SOURCE: (1) Department of Pharmacy, King's College London, Stamford  
Street, London, SE1 8WA UK

SOURCE: Biochemical Society Transactions, (October, 2000) Vol. 28,  
No. 5, pp. A435. print.

Meeting Info.: 18th International Congress of Biochemistry  
and Molecular Biology Birmingham, UK July 16-20, 2000

ISSN: 0300-5127.

DOCUMENT TYPE: Conference

LANGUAGE: English

SUMMARY LANGUAGE: English

L13 ANSWER 12 OF 42 MEDLINE

DUPLICATE 7

ACCESSION NUMBER: 2000476205 MEDLINE

DOCUMENT NUMBER: 20477761 PubMed ID: 11026737

09/583,310 Search Strategy/Results

TITLE: Cytochrome P-450 enzymes and FMO3 contribute to the disposition of the antipsychotic drug perazine in vitro.

AUTHOR: Stormer E; Brockmoller J; Roots I; Schmider J

CORPORATE SOURCE: Humboldt-University Berlin, Institute of Clinical Pharmacology, Germany.

SOURCE: PSYCHOPHARMACOLOGY, (2000 Sep) 151 (4) 312-20. Journal code: QGI. ISSN: 0033-3158.

PUB. COUNTRY: GERMANY: Germany, Federal Republic of Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200101

ENTRY DATE: Entered STN: 20010322  
Last Updated on STN: 20010322  
Entered Medline: 20010118

AB RATIONALE: Perazine (PER) is a phenothiazine antipsychotic drug frequently used in Germany that undergoes extensive metabolism. OBJECTIVES AND METHODS: To anticipate metabolic drug interactions and to explore the relevance of polymorphisms of metabolic enzymes, perazine-N-demethylation and perazine-N-oxidation were investigated in vitro using human liver microsomes and cDNA expressed enzymes. RESULTS: CYP3A4 and CYP2C9 were identified as the major enzymes mediating PER-N-demethylation. At 10 microM PER, a concentration consistent with anticipated in vivo liver concentrations, CYP3A4 and CYP2C9 contributed 50% and 35%, respectively, to PER-N-demethylation. With increasing PER concentrations, contribution of CYP2C9 decreased and CYP3A4 became more important. In human liver microsomes, PER-N-demethylation was inhibited by ketoconazole (>40%) and sulfaphenazole (16%). Allelic variants of recombinant CYP2C9 showed differences in PER-N-demethylase activity. The wild type allele CYP2C9\*1 was the most active variant. Maximal activities of CYP2C9\*2 and CYP2C9\*3 were 88% and 18%, respectively, compared to the wild type activity. Perazine-N-oxidation was mainly mediated by FMO3. In the absence of NADPH, heat treatment of microsomes abolished PER-N-oxidase activity. Methimazole inhibited PER-N-oxidation, while CYP specific inhibitors had no inhibitory effect. Perazine is a potent inhibitor of dextromethorphan-O-demethylase, S-mephenytoin-hydroxylase, alprazolam-4-hydroxylase, phenacetin-O-deethylase and tolbutamide-hydroxylase activity in human liver microsomes. CONCLUSIONS: Alterations in the activity of CYP3A4, CYP2C9 and FMO3 through genetic polymorphisms, enzyme induction or inhibition bear the potential to cause clinically significant changes in perazine clearance. PER may alter the clearance of coadministered compounds metabolized by CYP2D6, CYP2C19, CYP2C9, CYP3A4 and CYP1A2.

L13 ANSWER 13 OF 42 MEDLINE DUPLICATE 8

ACCESSION NUMBER: 2000501240 MEDLINE

DOCUMENT NUMBER: 20500167 PubMed ID: 11048672

TITLE: The house musk shrew (Suncus murinus): a unique animal with extremely low level of expression of mRNAs for CYP3A and flavin-containing monooxygenase.

AUTHOR: Mushiroda T; Yokoi T; Itoh K; Nunoya K; Nakagawa T; Kubota M; Takahara E; Nagata O; Kato H; Kamataki T

CORPORATE SOURCE: Division of Pharmacobio-dynamics, Graduate School of Pharmaceutical Sciences, Hokkaido University, Japan.. mushiroda@hokuriku-seiyaku.co.jp

SOURCE: COMPARATIVE BIOCHEMISTRY AND PHYSIOLOGY. TOXICOLOGY & PHARMACOLOGY, (2000 Jul) 126 (3) 225-34. Journal code: DTU. ISSN: 1532-0456.

PUB. COUNTRY: United States

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200102

ENTRY DATE: Entered STN: 20010322  
Last Updated on STN: 20010322  
Entered Medline: 20010201

AB Expression of drug-metabolizing enzymes including cytochrome P450 (CYP) and flavin-containing monooxygenase (FMO) in various tissues of Suncus murinus (Suncus) were examined. Northern blot analysis showed that mRNAs hybridizable with cDNAs for rat CYP1A2, human CYP2A6, rat CYP2B1, human CYP2C8, human CYP2D6, rat CYP2E1, human CYP3A4 and rat CYP4A1 were expressed in various tissues from Suncus. The mRNA level of CYP2A in the Suncus lung was very high. Furthermore, it was found that the level of CYP2A mRNA in the Suncus lung was higher compared to the Suncus liver. The expression level of mRNA hybridizable with cDNA for



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human CYP3A4 was very low. The presence of CYP3A gene in Suncus was proven by the induction of the CYP with dexamethasone. Very low expression levels of mRNAs hybridizable with cDNAs for rat FMO1, rat FMO2, rat FMO3 and rat FMO5 were also seen in Suncus liver. No apparent hybridization band appeared when human FMO4 cDNA was used as a probe. The hepatic expression of mRNAs hybridizable with cDNAs for UDP-glucuronosyltransferase 1\*6, aryl sulfotransferase, glutathione S-transferase 1, carboxylesterase and microsomal epoxide hydrolase in the Suncus were observed. These results indicate that the Suncus is a unique animal species in that mRNAs for CYP3A and FMO are expressed at very low levels.

L13 ANSWER 14 OF 42 MEDLINE DUPLICATE 9  
 ACCESSION NUMBER: 2000123346 MEDLINE  
 DOCUMENT NUMBER: 20123346 PubMed ID: 10659950  
 TITLE: Interspecies comparison and role of human cytochrome P450 and flavin-containing monooxygenase in hepatic metabolism of L-775,606, a potent 5-HT(1D) receptor agonist.  
 AUTHOR: Prueksaritanont T; Lu P; Gorham L; Sternfeld F; Vyas K P  
 CORPORATE SOURCE: Department of Drug Metabolism, Merck Research Laboratories, West Point, PA 19486, USA.. thomayant-prueksaritanont@merck.com  
 SOURCE: XENOBIOTICA, (2000 Jan) 30 (1) 47-59.  
 PUB. COUNTRY: ENGLAND: United Kingdom  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 200003  
 ENTRY DATE: Entered STN: 20000320  
 Last Updated on STN: 20000320  
 Entered Medline: 20000307

AB 1. Quantitative species differences and human liver enzymes involved in the metabolism of L-775,606, a potent and selective 5-HT1D receptor agonist developed for the acute treatment of migraine headache, have been investigated in vitro. 2. In human, monkey, dog and rat liver microsomes, formation of the hydroxylated M1 and the N-dealkylated M2 was mediated by enzyme(s) of high-affinity (apparent Km approximately 1-6 microM), and that of the two N-oxide isomers (M3) was catalysed by those of low affinity (apparent Km approximately 50-110 microM). In dog, M3 constituted a major pathway (approximately 40%), whereas in all other species it was a minor metabolite (< 5%). 3. In human liver microsomes, a marked inhibition (> or =80%) of M1 and M2 formation was observed by SKF525-A, troleandomycin, ketoconazole and anti-CYP3A antibodies, whereas the inhibition was modest (approximately 20-40%) with quercetin. Of seven cDNA-expressed human P450 tested, only CYP3A4 and CYP2C8 were capable of oxidizing L-775,606, resulting primarily in M1 and M2. However, CYP3A4 possessed much higher affinity (> or = 20-fold) and much higher intrinsic activity (> 100-fold) than CYP2C8. 4. In contrast, N-oxidation was not inhibited by any inhibitors of P450 tested, but rather was reduced significantly by heat treatment and methimazole, and was increased substantially with an incubation pH>7.4. Human flavin-containing monooxygenase form 3 (FMO3) catalysed exclusively the N-oxidation to M3, with apparent Km and optimum pH comparable with those observed in human liver microsomes. 5. These results demonstrated quantitative interspecies differences in the metabolism of L-775,606. In human, metabolism of L-775,606 to the principal metabolites, M1 and M2, was mediated primarily by CYP3A4 with minimal contribution from CYP2C8, whereas the minor N-oxidative pathway was catalysed mainly by FMO3.

L13 ANSWER 15 OF 42 MEDLINE DUPLICATE 10  
 ACCESSION NUMBER: 2000269720 MEDLINE  
 DOCUMENT NUMBER: 20269720 PubMed ID: 10807940  
 TITLE: Stereoselective sulfoxidation of sulindac sulfide by flavin-containing monooxygenases. Comparison of human liver and kidney microsomes and mammalian enzymes.  
 AUTHOR: Hamman M A; Haehner-Daniels B D; Wrighton S A; Rettie A E; Hall S D  
 CORPORATE SOURCE: Division of Clinical Pharmacology, Indiana University School of Medicine, Indianapolis, IN 46202, USA.  
 CONTRACT NUMBER: AG07631 (NIA)  
 SOURCE: GM43511 (NIGMS)  
 BIOCHEMICAL PHARMACOLOGY, (2000 Jul 1) 60 (1) 7-17.  
 Journal code: 9Z4; 0101032. ISSN: 0006-2952.

## 09/583,310 Search Strategy/Results

PUB. COUNTRY: ENGLAND: United Kingdom  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200006  
ENTRY DATE: Entered STN: 20000629  
Last Updated on STN: 20000629  
Entered Medline: 20000616

AB The stereoselective sulfoxidation of the pharmacologically active metabolite of sulindac, sulindac sulfide, was characterized in human liver, kidney, and cDNA-expressed enzymes. Kinetic parameter estimates (pH = 7.4) for sulindac sulfoxide formation in human liver microsomes (N = 4) for R- and S-sulindac sulfoxide were  $V(\max) = 1.5 \pm 0.50$  nmol/min/mg,  $K(m) = 15 \pm 5.1$  microM; and  $V(\max) = 1.1 \pm 0.36$  nmol/min/mg,  $K(m) = 16 \pm 6.1$  microM, respectively. Kidney microsomes (N = 3) produced parameter estimates (pH = 7.4) of  $V(\max) = 0.9 \pm 0.29$  nmol/min/mg,  $K(m) = 15 \pm 2.9$  microM;  $V(\max) = 0.5 \pm 0.21$  nmol/min/mg,  $K(m) = 22 \pm 1.9$  microM for R- and S-sulindac sulfoxide, respectively. In human liver and flavin-containing monooxygenase 3 (FMO3) the  $V(\max)$  for R-sulindac sulfoxide increased 60-70% at pH = 8.5, but for S-sulindac sulfoxide was unchanged. In fourteen liver microsomal preparations, significant correlations occurred between R-sulindac sulfoxide formation and either immunoquantified FMO or nicotine N-oxidation ( $r = 0.88$  and  $0.83$ ;  $P < 0.01$ ). The R- and S-sulindac sulfoxide formation rate also correlated significantly ( $r = 0.85$  and  $0.75$ ;  $P < 0.01$ ) with immunoquantified FMO in thirteen kidney microsomal samples. Mild heat deactivation of microsomes reduced activity by 30-60%, and a loss in stereoselectivity was observed. Methimazole was a potent and nonstereoselective inhibitor of sulfoxidation in liver and kidney microsomes. n-Octylamine and membrane solubilization with lubrol were potent and selective inhibitors of S-sulindac sulfoxide formation. cDNA-expressed CYPs failed to appreciably sulfoxidate sulindac sulfide, and CYP inhibitors were ineffective in suppressing catalytic activity. Purified mini-pig liver FMO1, rabbit lung FMO2, and human cDNA-expressed FMO3 efficiently oxidized sulindac sulfide with a high degree of stereoselectivity towards the R-isomer, but FMO5 lacked catalytic activity. The biotransformation of the sulfide to the sulfoxide is catalyzed predominately by FMOs and may prove to be useful in characterizing FMO activity.

L13 ANSWER 16 OF 42 MEDLINE DUPLICATE 11  
ACCESSION NUMBER: 1999268413 MEDLINE  
DOCUMENT NUMBER: 99268413 PubMed ID: 10338091  
TITLE: Two novel mutations of the FMO3 gene in a proband with trimethylaminuria.  
AUTHOR: Akerman B R; Forrest S; Chow L; Youil R; Knight M; Treacy E P  
CORPORATE SOURCE: C.R. Scriver Biochemical Genetics Unit, Montreal Children's Hospital, Quebec, Canada.  
SOURCE: HUMAN MUTATION, (1999) 13 (5) 376-9.  
JOURNAL CODE: BRD; 9215429. ISSN: 1059-7794.  
PUB. COUNTRY: United States  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
OTHER SOURCE: GENBANK-U39960; GENBANK-U39961; GENBANK-U39962;  
GENBANK-U39963; GENBANK-U39964; GENBANK-U39965;  
GENBANK-U39966; GENBANK-U39967  
ENTRY MONTH: 199908  
ENTRY DATE: Entered STN: 19990820  
Last Updated on STN: 19990820  
Entered Medline: 19990811

AB The mammalian flavin-containing monooxygenases catalyze the NADPH-dependent N-oxygenation of nucleophilic nitrogen-, sulfur-, and phosphorus-containing chemicals, drugs, and xenobiotics, including trimethylamine. The FMO3 gene encodes the dominant catalytically active isoform present in human liver. We have identified two missense mutations in the coding region of the gene in a proband with trimethylaminuria (TMA): M66I and R492W. Whereas two mutations (P153L, E305X) accounted for TMA in our eight unrelated previously documented Australian families of British origin, the present report is the first evidence of compound heterozygosity for two rare mutations in a proband with this disorder. This suggests that other rarer alleles, also causing TMA, will be found in the same populations.

L13 ANSWER 17 OF 42 MEDLINE DUPLICATE 12  
ACCESSION NUMBER: 1999102145 MEDLINE

## 09/583,310 Search Strategy/Results

DOCUMENT NUMBER: 99102145 PubMed ID: 9884321  
TITLE: In vitro metabolism of the M1-muscarinic agonist  
5-(2-ethyl-2H-tetrazol-5-yl)-1-methyl-1,2,3,6-  
tetrahydropyridine by human hepatic cytochromes  
P-450 determined at pH 7.4 and 8.5.  
AUTHOR: Jensen K G; Dalgaard L  
CORPORATE SOURCE: Department of Drug Metabolism, H. Lundbeck A/S, Ottiliavej  
9, Copenhagen-Valby, Denmark.. KGJ@Lundbeck.com  
SOURCE: DRUG METABOLISM AND DISPOSITION, (1999 Jan) 27 (1) 125-32.  
Journal code: EBR; 9421550. ISSN: 0090-9556.  
PUB. COUNTRY: United States  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199903  
ENTRY DATE: Entered STN: 19990316  
Last Updated on STN: 19990316  
Entered Medline: 19990301

AB Biotransformation of the M1-muscarinic agonist Lu 25-109  
(5-(2-ethyl-2H-tetrazol-5-yl)-1-methyl-1,2,3,6-tetrahydropyridine) , in  
development for the treatment of Alzheimer's disease, was investigated to  
obtain information on the identity of human hepatic cytochrome  
P-450 enzymes involved in its metabolism. The identification of these  
P-450s was accomplished through studies using 1) simple regression  
analysis with 14 phenotyped human liver samples, 2)  
selective chemical inhibitors, and 3) microsomes containing cDNA  
-expressed enzymes. The production of some metabolites is enhanced in  
vitro when the pH of the incubation media is shifted from pH 7.4 to 8.5.  
The metabolite production in human liver microsomes  
was NADPH-dependent, suggesting that the metabolism of Lu 25-109 in  
human liver microsomes is primarily P-450-dependent. Lu  
25-109 was metabolized by human liver microsomes to Lu  
31-126 (de-ethyl Lu 25-109) mainly by CYP2D6; to Lu 29-297  
[3-(2-ethyltetrazol-5-yl)-1-methyl-pyridinium] and Lu 25-077 (demethyl Lu  
25-109) mainly by CYP1A2, CYP2A6, CYP2C19, and CYP3A4; and to Lu 32-181  
(Lu 25-109 N-oxide) by CYP1A2 and possibly by CYP2C19. One metabolite, Lu  
32-181 (N-oxide), could be reduced back to Lu 25-109, a reaction not  
inhibited by the applied cytochrome P-450 inhibitors. This study did not  
indicate any involvement of FMO3 or MAO in the in vitro  
metabolism of Lu 25-109 in human liver microsomes.

L13 ANSWER 18 OF 42 MEDLINE DUPLICATE 13  
ACCESSION NUMBER: 97426128 MEDLINE  
DOCUMENT NUMBER: 97426128 PubMed ID: 9282832  
TITLE: Detoxication of tyramine by the flavin-containing  
monooxygenase: stereoselective formation of the trans  
oxime.  
AUTHOR: Lin J; Cashman J R  
CORPORATE SOURCE: Seattle Biomedical Research Institute, Washington 98109,  
USA.  
CONTRACT NUMBER: 00269 (NIDA)  
DA 08531 (NIGMS)  
GM 36426  
+  
SOURCE: CHEMICAL RESEARCH IN TOXICOLOGY, (1997 Aug) 10 (8) 842-52.  
Journal code: ASX; 8807448. ISSN: 0893-228X.  
PUB. COUNTRY: United States  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199710  
ENTRY DATE: Entered STN: 19971013  
Last Updated on STN: 19971013  
Entered Medline: 19971001

AB In the presence of pig or adult human liver  
microsomes, tyramine was metabolized to the corresponding trans oxime  
through the intermediacy of the hydroxylamine. The requisite intermediate,  
(4-hydroxyphenethyl)hydroxylamine, was retroreduced to tyramine or  
converted stereoselectively to the trans oxime in the presence of pig or  
adult human liver microsomes. Studies of the effect of  
metabolic inhibitors suggested that formation of the trans oxime and  
retroreduction of the hydroxylamine were largely dependent on NADPH and  
the flavin-containing monooxygenase (FMO) and cytochrome P450,  
respectively. The conclusion that FMO was predominantly responsible for  
trans oxime formation in human liver microsomes was  
based on the effect of incubation conditions on tyramine N-oxygenation and  
the observation that cDNA-expressed human FMO3  
also N-oxygenated tyramine to give exclusively the trans oxime. The

synthetic hydroxylamine and oxime metabolites of tyramine were examined for affinity to human and animal dopamine and serotonin receptors and the human dopamine transporter. For all of the receptors and for the transporter examined, the avidity of the hydroxylamine and oximes was greater than 10 microM and beyond the effective concentration for physiological relevance. The results suggested that tyramine was sequentially N-oxygenated in the presence of pig and human liver microsomes and cDNA-expressed FMO3 to the hydroxylamine and then to the di-N-hydroxylamine that was spontaneously dehydrated to the trans oxime. This may be facilitated by FMO through a nondissociative substrate-enzyme interaction. Based on the biogenic amine receptor or transporter affinity for the hydroxylamine and oxime metabolites of tyramine, N-oxygenation of tyramine by pig or human liver FMO may represent a detoxication reaction that terminates the pharmacological activity of tyramine.

L13 ANSWER 19 OF 42 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 97227540 EMBASE

DOCUMENT NUMBER: 1997227540

TITLE: Baculovirus-mediated expression and purification of human FMO3: Catalytic, immunochemical, and structural characterization.

AUTHOR: Haining R.L.; Hunter A.P.; Sadeque A.J.M.; Philpot R.M.; Rettie A.E.

CORPORATE SOURCE: A.E. Rettie, Department of Medicinal Chemistry, Box 357610, University of Washington, Seattle, WA 98195, United States

SOURCE: Drug Metabolism and Disposition, (1997) 25/7 (790-797).

Refs: 33

ISSN: 0090-9556 CODEN: DMDSAI

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 029 Clinical Biochemistry

LANGUAGE: English

SUMMARY LANGUAGE: English

AB The baculovirus expression vector system was used to overexpress human FMO3 in insect cells for catalytic, structural, and immunochemical studies. Membranes prepared from infected *Trichoplusia ni* cell suspensions catalyzed NADPH-dependent metabolism of methyl p-tolyl sulfide at rates 20 times faster than those obtained with detergent-solubilized human liver microsomes. Sulfoxidation of the methyl and ethyl p-tolyl sulfides by recombinant human FMO3 proceeded with little stereochemical preference, whereas sulfoxidation of the n-propyl and n-butyl homologs demonstrated increasing selectivity for formation of the (R)-sulfoxide. This chiral fingerprint recapitulated the metabolite profile obtained when detergent-treated human liver microsomes served as the enzyme source. Catalytically active human FMO3 was purified to apparent homogeneity by cholate solubilization and sequential column chromatography on Octyl-Sepharose, DEAE-Sepharose, and hydroxyapatite. Purified FMO3 exhibited the same electrophoretic mobility as native microsomal enzyme, and immunoquantitation showed that this isoform represents approx. 0.5% of human liver microsomal protein. Therefore, FMO3 is quantitatively a major human liver monooxygenase. LC/electrospray-mass spectrometry analysis of purified FMO3 identified >70% of the tryptic peptides, including fragments containing motifs for N-linked glycosylation and O-linked glycosylation. Although insect cells have the capacity for glycan modification, MS analysis of the tryptic peptides demonstrated that these sites were not modified in the purified, recombinant enzyme. Edman degradation of the recombinant product revealed that posttranslational modification of human FMO3 by insect cells was limited to cleavage at the N-terminal methionine, a process seen in vivo with animal orthologs of FMO3. These studies demonstrate the suitability of this eukaryotic system for heterologous expression of human FMOs and future detailed analysis of their substrate specificities.

L13 ANSWER 20 OF 42 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 1997:486050 BIOSIS

DOCUMENT NUMBER: PREV199799785253

TITLE: Determination of FAD-binding domain in flavin-containing monooxygenase 1 (FMO1).

AUTHOR(S): Kubo, Akiko; Itoh, Susumu (1); Itoh, Kunio; Kamataki, Tetsuya

CORPORATE SOURCE: (1) Ludwig Inst. Cancer Res., Box 595, S-751 24 Uppsala Sweden

SOURCE: Archives of Biochemistry and Biophysics, (1997) Vol. 345, No. 2, pp. 271-277.

ISSN: 0003-9861.

DOCUMENT TYPE: Article

LANGUAGE: English

AB The flavin-containing monooxygenases (FMOs) are a family of flavoenzymes and contain one molecule of FAD per monomer. In order to demonstrate where FMO interacts with FAD, four mutants for the rat liver FMO1 protein were expressed in yeast and characterized. All four mutants were immunochemically similar to the unmodified form, although the contents of FAD in all four mutants were much lower than that in the unmodified form. Interestingly, the mutant generated by changing the first glycine of the proposed FAD-binding domain (GxGxxG) to alanine revealed catalytic activities, but was lower than those seen with the unmodified form. The conversion of the first glycine to alanine markedly increased and decreased the K-m and V-max values for imipramine N-oxidation, respectively. The other three mutants (RFMOM2, RFMOM3, and RFMOM4) were catalytically inactive. Our results suggest that three glycines, especially the second and third glycines, in the proposed FAD-binding domain were necessary for FMO to show catalytic activities. Using RFMOM1 and the unmodified form, the effects of n-octylamine on the activity of FMO1 were investigated. The activities of both wild-type and RFMOM1 enzymes for all of the compounds examined were enhanced by n-octylamine. The K-m and V-max values of both RFMOM1 and the unmodified form for imipramine N-oxidation were lowered and raised by n-octylamine, respectively.

L13 ANSWER 21 OF 42 MEDLINE DUPLICATE 14  
ACCESSION NUMBER: 1998086483 MEDLINE  
DOCUMENT NUMBER: 98086483 PubMed ID: 9417913  
TITLE: Structural organization of the human  
flavin-containing monooxygenase 3 gene (FMO3),  
the favored candidate for fish-odor syndrome, determined  
directly from genomic DNA.  
AUTHOR: Dolphin C T; Riley J H; Smith R L; Shephard E A; Phillips I  
R  
CORPORATE SOURCE: Department of Biochemistry, Queen Mary & Westfield College,  
University of London, United Kingdom.  
SOURCE: GENOMICS, (1997 Dec 1) 46 (2) 260-7.  
Journal code: GEN; 8800135. ISSN: 0888-7543.  
PUB. COUNTRY: United States  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
OTHER SOURCE: GENBANK-U39960; GENBANK-U39961; GENBANK-U39962;  
GENBANK-U39963; GENBANK-U39964; GENBANK-U39965;  
GENBANK-U39966; GENBANK-U39967  
ENTRY MONTH: 199802  
ENTRY DATE: Entered STN: 19980217  
Last Updated on STN: 19980217  
Entered Medline: 19980203

AB The inherited metabolic disorder trimethylaminuria (fish-odor syndrome) is associated with defective hepatic N-oxidation of dietary-derived trimethylamine catalyzed by flavin-containing monooxygenase (FMO). As FMO3 encodes the major form of FMO expressed in adult human liver, it represents the best candidate gene for the disorder. The structural organization of FMO3 was determined by sequencing the products of exon-to-exon and vectorette PCR, the latter through the use of vectorette libraries constructed directly from genomic DNA. The gene contains one noncoding and eight coding exons. Knowledge of the exon/intron organization of the human FMO3 gene enabled each of the coding exons of the gene, together with their associated flanking intron sequences, to be amplified from genomic DNA and will thus facilitate the identification of mutations in FMO3 in families affected with fish-odor syndrome.

L13 ANSWER 22 OF 42 MEDLINE DUPLICATE 15  
ACCESSION NUMBER: 97231261 MEDLINE  
DOCUMENT NUMBER: 97231261 PubMed ID: 9076656  
TITLE: Molecular cloning of mouse liver flavin  
containing monooxygenase (FMO1) cDNA and  
characterization of the expression product: metabolism of  
the neurotoxin, 1,2,3,4-tetrahydroisoquinoline (TIQ).  
AUTHOR: Itoh K; Nakamura K; Kimura T; Itoh S; Kamataki T  
CORPORATE SOURCE: Division of Drug Metabolism, Faculty of Pharmaceutical  
Sciences, Hokkaido University, Japan.  
SOURCE: JOURNAL OF TOXICOLOGICAL SCIENCES, (1997 Feb) 22 (1) 45-56.  
Journal code: KAE; 7805798. ISSN: 0388-1350.  
PUB. COUNTRY: Japan  
Journal; Article; (JOURNAL ARTICLE)

09/583,310 Search Strategy/Results

LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199705  
 ENTRY DATE: Entered STN: 19970609  
 Last Updated on STN: 19970609  
 Entered Medline: 19970529

AB A mouse liver cDNA clone, FMO1, coding for a flavin-containing monooxygenase (FMO) was isolated. This cDNA clone encoded a protein of 532 amino acids. Based upon its predicted amino acid sequence, this clone was assumed to belong to the FMO1 subfamily. The deduced amino acid sequence showed 94, 84, 83, and 83% identity with FMO1s of rats, pigs, rabbits and humans, respectively, while it showed only 50-59% identity with human FMO3 and FMO4, rabbit FMO2, FMO3, FMO4 and FMO5, and guinea-pig FMO2. RNA blot analysis showed that the mouse FMO1 was also expressed in the lung and kidney and to lesser extents in the heart, spleen, testis and brain. Mouse FMO1 expressed in yeast showed activities of thiobenzamide S-oxidation, and NADPH oxidation associated with the S- or N-oxidation of chlorpromazine, N,N-dimethylaniline, N,N-dimethyl-hydrazine, imipramine, nicotine, thioacetamide, thiourea and trimethylamine. Moreover, 1,2,3,4-tetrahydroisoquinoline (TIQ), a substance known to induce a parkinsonism-like syndrome in monkeys, was also metabolized by the mouse FMO1. The K(m) values for chlorpromazine, imipramine and TIQ were determined to be 2.4, 16.0, 435 mM, respectively. This is the first report to show that an expressed FMO can metabolize a neurotoxin, TIQ.

L13 ANSWER 23 OF 42 MEDLINE

ACCESSION NUMBER: 97450419 MEDLINE  
 DOCUMENT NUMBER: 97450419 PubMed ID: 9305407  
 TITLE: Quantitation and kinetic properties of hepatic microsomal and recombinant flavin-containing monooxygenases 3 and 5 from humans.  
 AUTHOR: Overby L H; Carver G C; Philpot R M  
 CORPORATE SOURCE: Laboratory of Signal Transduction, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709, USA.  
 SOURCE: CHEMICO-BIOLOGICAL INTERACTIONS, (1997 Aug 29) 106 (1) 29-45.  
 Journal code: CYV; 0227276. ISSN: 0009-2797.  
 PUB. COUNTRY: Ireland  
 Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199710  
 ENTRY DATE: Entered STN: 19971024  
 Last Updated on STN: 19971024  
 Entered Medline: 19971016

AB Variable amounts of flavin-containing monooxygenase isoforms 3 and 5 (FMO3 and FMO5) are present in microsomal preparations from adult, male, human liver. Quantitation with monospecific antibodies and recombinant isoforms as standards showed levels of FMO3 and of FMO5 that ranged from 12.5 to 117 and 3.5 to 34 pmol/mg microsomal protein, respectively. The concentration of FMO3 was greater than that of FMO5 in all samples, but the ratio of FMO3 to FMO5 varied from 2:1 to 10:1. Human hepatic microsomal samples also showed variable activities for the S-oxidation of methimazole. This activity was associated totally with FMO3; no participation of FMO5 was apparent. This conclusion was supported by several lines of evidence: first, the catalytic efficiency of FMO3 with methimazole was found to be approximately 5000 times greater than that of FMO5; second, the rate of metabolism showed a direct, quantitative relationship with FMO3 content; third, the plot of the relationship between metabolism and FMO3 content extrapolated close to the origin. A second reaction, the N-oxidation of ranitidine, exhibited a much higher Km with recombinant FMO3 than did methimazole (2 mM vs. 35 microM). However, a direct relationship between this reaction and FMO3 content in human hepatic microsomal preparations was also apparent. This result shows that even with a high Km substrate, FMO3-catalyzed metabolism can account for the majority of the product formation with some drugs. Our findings demonstrate that the contribution of FMO isoforms to human hepatic drug metabolism can be assessed quantitatively on the basis of the characteristics of the enzymes expressed in Escherichia coli.

L13 ANSWER 24 OF 42 MEDLINE

ACCESSION NUMBER: 1998008021 MEDLINE  
 DOCUMENT NUMBER: 98008021 PubMed ID: 9344459  
 TITLE: Molecular cloning, sequencing, and expression in

DUPLICATE 16

09/583,310 Search Strategy/Results

Escherichia coli of mouse flavin-containing monooxygenase 3 (FMO3): comparison with the human isoform.

AUTHOR: Falls J G; Cherrington N J; Clements K M; Philpot R M; Levi P E; Rose R L; Hodgson E

CORPORATE SOURCE: Department of Toxicology, North Carolina State University, Raleigh, North Carolina 27695, USA.

CONTRACT NUMBER: ES00044 (NIEHS)

SOURCE: ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, (1997 Nov 1) 347 (1) 9-18.

PUB. COUNTRY: Journal code: 6SK; 0372430. ISSN: 0003-9861. United States

LANGUAGE: Journal; Article; (JOURNAL ARTICLE) English

FILE SEGMENT: Priority Journals

OTHER SOURCE: GENBANK-U87147

ENTRY MONTH: 199712

ENTRY DATE: Entered STN: 19980109  
Last Updated on STN: 19980109  
Entered Medline: 19971202

AB The sequence of mouse flavin-containing monooxygenase 3 (FMO3) was obtained from several clones isolated from a mouse liver cDNA library. The nucleotide sequence of mouse FMO3 was 2020 bases in length containing 37 bases in the 5' flanking region, 1602 in the coding region, and 381 in the 3' flanking region. The derived protein sequence consisted of 534 amino acids including the putative flavin adenine dinucleotide and NADP+ pyrophosphate binding sites (characteristic of mammalian FMOs) starting at positions 9 and 191, respectively. The mouse FMO3 protein sequence was 79 and 82% identical to the human and rabbit FMO3 sequences, respectively. Mouse FMO3 was expressed in Escherichia coli and compared to E. coli expressed human FMO3. The FMO3 proteins migrated with the same mobility (approximately 58 kDa) as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting. The expressed FMO3 enzymes (mouse and human forms) were sensitive to heat and reacted in a similar manner toward metal ions and detergent. Catalytic activities of mouse and human FMO3 were high toward the substrate methimazole; however, in the presence of trimethylamine and thioacetamide, FMO-dependent methimazole oxidation by both enzymes was reduced by greater than 85%. Other substrates which inhibited methimazole oxidation were thiourea and thiobenzamide and to a lesser degree N,N-dimethylaniline. When probed with mouse FMO3 cDNA, FMO3 transcripts were detected in hepatic mRNA samples from female mice, but not in samples from males. FMO3 was detected in mRNA samples from male and female mouse lung, but FMO3 message was not detected in mouse kidney sample from either gender. Results of immunoblotting confirmed the tissue- and gender-dependent expression of mouse FMO3.

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L13 ANSWER 25 OF 42 MEDLINE DUPLICATE 17

ACCESSION NUMBER: 97057945 MEDLINE

DOCUMENT NUMBER: 97057945 PubMed ID: 8902275

TITLE: N-oxygenation of primary amines and hydroxylamines and retroreduction of hydroxylamines by adult human liver microsomes and adult human flavin-containing monooxygenase 3.

AUTHOR: Lin J; Berkman C E; Cashman J R

CORPORATE SOURCE: Seattle Biomedical Research Institute, Washington 98108, USA.

CONTRACT NUMBER: GM36426 (NIGMS)

SOURCE: CHEMICAL RESEARCH IN TOXICOLOGY, (1996 Oct-Nov) 9 (7) 1183-93.

PUB. COUNTRY: Journal code: A5X; 8807448. ISSN: 0893-228X. United States

LANGUAGE: Journal; Article; (JOURNAL ARTICLE) English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199702

ENTRY DATE: Entered STN: 19970305  
Last Updated on STN: 19970305  
Entered Medline: 19970220

AB Adult human liver microsomes catalyze the NADPH-dependent N-oxygenation of 10-N-(n-octylamino)-2-(trifluoromethyl)phenothiazine to the corresponding oximes through the intermediacy of the hydroxylamine. In the presence of adult human liver microsomes, the primary amine is stereoselectively converted

to the cis-oxime, but addition of the alternative competitive substrate hydroxylamine hydrochloride apparently decreases the amount of aliphatic hydroxylamine retroreduction because an increase in oxime formation was observed. In the presence of hydroxylamine hydrochloride, however, the oxime product recovered was formed with very low stereoselectivity. Studies on the biochemical mechanism of oxime formation suggested that cis-oxime formation in the presence of adult human liver microsomes was largely dependent on the human flavin-containing monooxygenase (form 3). This conclusion is based on the effects of incubation conditions on product formation when compared to results observed in the presence of cDNA-expressed human FMO3. The retroreduction of the intermediate hydroxylamine was dependent on NADPH but was not catalyzed by human flavin-containing monooxygenase (form 3) or any one of seven prominent cytochromes P-450 that have been well-characterized in the human liver microsomes examined. The results suggest that aliphatic primary amines are efficiently sequentially N-oxygenated in the presence of human liver microsomes to hydroxylamines and then to oximes mainly by the human flavin-containing monooxygenase. Retroreduction of the intermediate hydroxylamine is apparently facilitated by a novel but as yet poorly characterized enzyme system that does not employ any of the currently known well-characterized cytochrome P-450 enzymes present in adult human liver microsomes.

L13 ANSWER 26 OF 42 MEDLINE DUPLICATE 18  
 ACCESSION NUMBER: 96184548 MEDLINE  
 DOCUMENT NUMBER: 96184548 PubMed ID: 8654418  
 TITLE: Differential developmental and tissue-specific regulation of expression of the genes encoding three members of the flavin-containing monooxygenase family of man, FMO1, FMO3 and FMO4.  
 AUTHOR: Dolphin C T; Cullingford T E; Shephard E A; Smith R L; Phillips I R  
 CORPORATE SOURCE: Department of Biochemistry, Queen Mary & Westfield College, University of London, UK.  
 SOURCE: EUROPEAN JOURNAL OF BIOCHEMISTRY, (1996 Feb 1) 235 (3) 683-9.  
 PUB. COUNTRY: JOURNAL code: EMZ; 0107600. ISSN: 0014-2956.  
 LANGUAGE: ENGLISH  
 FILE SEGMENT: PRIORITY JOURNALS  
 OTHER SOURCE: GENBANK-Z47552  
 ENTRY MONTH: 199607  
 ENTRY DATE: Entered STN: 19960808  
 Last Updated on STN: 19960808  
 Entered Medline: 19960730

AB We have previously described the isolation and sequencing of cDNA clones encoding flavin-containing monooxygenases (FMOs) 1 and 4 of man [Dolphin, C., Shephard, E. A., Povey, S., Palmer, C. N. A., Ziegler, D. M., Ayesh, R., Smith, R. L. & Phillips, I. R. (1991) J. Biol. Chem. 266, 12379-12385; Dolphin, C., Shephard E. A., Povey, S., Smith, R. L. & Phillips, I. R. (1992) Biochem. J. 287, 261-267]. We present here the isolation of a cDNA for FMO3 of man. The sequence of this cDNA and the amino acid sequence deduced from it differ substantially from those previously reported for this member of the FMO family of man. In addition, we have investigated, by quantitative RNase protection assays, the expression in several foetal and adult human tissues of genes encoding FMO1, FMO3 and FMO4. Our results demonstrate that, in the adult, FMO1 is expressed in kidney but not in liver, whereas in the foetus it is expressed in both organs. The lack of expression of FMO1 in adult human liver is in marked contrast to the situation in other mammals, such as pig and rabbit, in which FMO1 constitutes a major form of the enzyme in the liver of the adult animal. The mRNA encoding FMO3 is abundant in adult liver and is also present, in low abundance, in some foetal tissues. Thus, FMO1 and FMO3 are both subject to developmental and tissue-specific regulation, with a developmental switch in the expression of the genes taking place in the liver. FMO4 mRNA is present in low abundance in several foetal and adult tissues and thus the corresponding gene appears to be expressed constitutively.

L13 ANSWER 27 OF 42 MEDLINE DUPLICATE 19  
 ACCESSION NUMBER: 96223482 MEDLINE  
 DOCUMENT NUMBER: 96223482 PubMed ID: 8632334  
 TITLE: Identification of the human cytochromes P450 responsible for the in vitro formation of the major



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oxidative metabolites of the antipsychotic agent olanzapine.  
 AUTHOR: Ring B J; Catlow J; Lindsay T J; Gillespie T; Roskos L K; Cerimele B J; Swanson S P; Hamman M A; Wrighton S A  
 CORPORATE SOURCE: Department of Drug Metabolism and Disposition, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana, USA.  
 SOURCE: JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, (1996 Feb) 276 (2) 658-66.  
 Journal code: JP3; 0376362. ISSN: 0022-3565.  
 PUB. COUNTRY: United States  
 Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199607  
 ENTRY DATE: Entered STN: 19960715  
 Last Updated on STN: 19960715  
 Entered Medline: 19960703

AB The formation kinetics of 2-hydroxymethyl olanzapine (2-OH olanzapine), 4'-N-oxide olanzapine (N-O olanzapine) and 4'-N-desmethyl olanzapine (NdM olanzapine) were analyzed in vitro. Biphasic kinetics were observed for formation of 2-OH and NdM olanzapine. The high-affinity enzyme responsible for 2-OH olanzapine formation by two human liver samples exhibited an intrinsic clearance (CL<sub>int</sub>) of 0.2 microliter/min/mg. NdM olanzapine formation by two human liver samples exhibited a CL<sub>int</sub> of 1.0 microliter/min/mg for the high affinity enzyme. The formation of N-O olanzapine was linear up to 300 microM olanzapine, yielding a CL<sub>int</sub> of 0.32 to 1.70 microliters/min/mg. The formation of 7-hydroxy olanzapine (7-OH olanzapine) exhibited an apparent K<sub>m</sub> of 24.2 microM. The rates of 2-OH olanzapine formation correlated with CYP2D6 levels and activity, and it was formed to the greatest extent by cDNA-expressed CYP2D6. N-O olanzapine formation correlated with human liver flavin-containing monooxygenase (FMO3) levels and activity. NdM olanzapine and 7-OH olanzapine formation correlated with CYP1A2 catalytic activities and they were formed to the greatest extent by expressed CYP1A2. These results suggest that CYP1A2 catalyzes NdM olanzapine and 7-OH olanzapine formation, CYP2D6 catalyzes 2-OH olanzapine formation and FMO3 catalyzes N-O olanzapine formation.

L13 ANSWER 28 OF 42 MEDLINE

ACCESSION NUMBER: 96374838 MEDLINE  
 DOCUMENT NUMBER: 96374838 PubMed ID: 8786146  
 TITLE: Localization of human flavin-containing monooxygenase genes FMO2 and FMO5 to chromosome 1q.  
 AUTHOR: McCombie R R; Dolphin C T; Povey S; Phillips I R; Shephard E A  
 CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, University College London, Gower Street, London, WC1E 6BT, United Kingdom.  
 SOURCE: GENOMICS, (1996 Jun 15) 34 (3) 426-9.  
 Journal code: GEN; 8800135. ISSN: 0888-7543.  
 PUB. COUNTRY: United States  
 Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199609  
 ENTRY DATE: Entered STN: 19961008  
 Last Updated on STN: 19961008  
 Entered Medline: 19960924

AB The human flavin-containing monooxygenase (FMO) gene family comprises at least five distinct members (FMO1 to FMO5) that code for enzymes responsible for the oxidation of a wide variety of soft nucleophilic substrates, including drugs and environmental pollutants. Three of these genes (FMO1, FMO3, and FMO4) have previously been localized to human chromosome 1q, raising the possibility that the entire gene family is clustered in this chromosomal region. Analysis by polymerase chain reaction of DNA isolated from a panel of human-rodent somatic cell hybrids demonstrates that the two remaining identified members of the FMO gene family, FMO2 and FMO5, also are located on chromosome 1q.

L13 ANSWER 29 OF 42 MEDLINE

ACCESSION NUMBER: 96115258 MEDLINE  
 DOCUMENT NUMBER: 96115258 PubMed ID: 8654204  
 TITLE: In vitro hepatic metabolism of ABT-418 in chimpanzee (Pan troglodytes). A unique pattern of microsomal flavin-containing monooxygenase-dependent stereoselective

DUPLICATE 20

09/583,310 Search Strategy/Results

N'-oxidation.  
 AUTHOR: Rodrigues A D; Kukulka M J; Ferrero J L; Cashman J R  
 CORPORATE SOURCE: Drug Metabolism Department, Abbott Laboratories, Abbott  
 Park, IL 60064-3500, USA.  
 CONTRACT NUMBER: GM36426 (NIGMS)  
 SOURCE: DRUG METABOLISM AND DISPOSITION, (1995 Oct) 23 (10)  
 1143-52.  
 Journal code: EBR; 9421550. ISSN: 0090-9556.  
 PUB. COUNTRY: United States  
 Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199607  
 ENTRY DATE: Entered STN: 19960808  
 Last Updated on STN: 19980206  
 Entered Medline: 19960726

AB Metabolism of the cholinergic channel activator [N-methyl-3H]ABT-418 was studied using precision-cut tissue slices and microsomes (+/- cytosol) prepared from a single chimpanzee liver. In both cases, the products of C-oxidation (lactam) and N'-oxidation (cis > trans) were detected. In the presence of chimpanzee liver microsomes and cytosol, which had been characterized with respect to the levels of aldehyde oxidase (N1-methylnicotinamide oxidase), NADPH-dependent flavin-containing monooxygenase (FMO; N, N-dimethylaniline N-oxidase), and various cytochrome P450 (CYP)-dependent monooxygenase activities, ABT-418 lactam and N'-oxide formation was found to be largely dependent on CYP/aldehyde oxidase and FMO, respectively. The rank order of total (trans + cis) FMO-dependent N'-oxidation in liver microsomes was dog > rat > rabbit > chimpanzee > or = cynomolgus monkey > human. It is concluded that the metabolic profile of ABT-418 in the chimpanzee is unique. First, the C-/N'-oxidation ratio in liver slices (0.43) is similar to that of the rat and dog and dissimilar to that of the rat and dog and dissimilar to that of the two other primate species studied; human and cynomolgus monkey (C-/N'-oxidation ratio > or = 9.4). Second, the pattern of ABT-418 N'-oxidation observed with chimpanzee liver microsomes, and liver slices (trans:cis = 1:3), differs from that of rat, rabbit, and dog liver microsomes, rat and human kidney S-9 (trans >> cis), human liver microsomes (trans:cis approximately 1:1), and cynomolgus monkey (trans:cis approximately 2:1) liver microsomes. Lack of stereoselective N'-oxidation by human FMO was confirmed with cDNA-expressed FMO3.

L13 ANSWER 30 OF 42 MEDLINE DUPLICATE 21

ACCESSION NUMBER: 95374576 MEDLINE  
 DOCUMENT NUMBER: 95374576 PubMed ID: 7646564  
 TITLE: In vitro-in vivo correlations of human  
 (S)-nicotine metabolism.  
 AUTHOR: Berkman C E; Park S B; Wrighton S A; Cashman J R  
 CORPORATE SOURCE: Seattle Biomedical Research Institute, WA 98109, USA.  
 CONTRACT NUMBER: TT016614  
 SOURCE: BIOCHEMICAL PHARMACOLOGY, (1995 Aug 8) 50 (4) 565-70.  
 Journal code: 9Z4; 0101032. ISSN: 0006-2952.  
 PUB. COUNTRY: ENGLAND: United Kingdom  
 Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199509  
 ENTRY DATE: Entered STN: 19950930  
 Last Updated on STN: 19950930  
 Entered Medline: 19950920

AB The profile of (S)-nicotine metabolism in human liver microsomes was examined at concentrations approaching in vivo conditions (10 microm). At such concentrations, no (S)-nicotine N-1'-oxygenation was seen, and thus C-oxidation to the (S)-nicotine delta 1',5'-iminium ion was the sole product observed in the metabolic profile in the presence of the human liver microsomes. For simplicity of analysis, the (S)-nicotine delta 1',5'-iminium ion formed was converted to (S)-cotinine in the presence of exogenously added aldehyde oxidase. To explain the lack of (S)-nicotine N-1'-oxygenation at low (S)-nicotine concentrations, inhibition of flavin-containing monooxygenase (FMO) activity by (S)-cotinine was examined. Although (S)-cotinine was observed to inhibit pig FMO1 (Ki = 675 microm), partially purified cDNA-expressed adult human liver FMO3 was not inhibited by (S)-cotinine. We therefore concluded that the kinetic properties of the nicotine N'- and C-oxidases were responsible for the metabolic product profile observed. Kinetic constants were determined for individual human liver microsomal preparations from low (10 microm)

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and high (500 microm) (S)-nicotine concentrations by monitoring (S)-cotinine formation with HPLC. The mean  $K_{mapp}$  and  $V_{max}$  for formation of (S)-cotinine by the microsomes examined were 39.6 microm and 444.3 pmol.min<sup>-1</sup>.(mg protein)<sup>-1</sup>, respectively. The formation of (S)-cotinine was strongly dependent on the previous drug administration history of each subject, and among the highest rates for (S)-cotinine formation were those of the barbiturate-pretreated subjects. The rate of (S)-cotinine formation at low (10 microm) concentration correlated well with immunoreactivity for cytochrome P450 2A6 ( $r = 0.89$ ). In vitro-in vivo correlation of the results suggests that the low amount of (S)-nicotine N-1'-oxygenation and the large amount of (S)-cotinine formed in human smokers (i.e. 4 and 30% of a typical dose, respectively) are determined primarily by the kinetic properties of the human monooxygenase enzyme systems. However, additional non-hepatic monooxygenase(s) contributes to (S)-nicotine metabolism.

L13 ANSWER 31 OF 42 MEDLINE DUPLICATE 22

ACCESSION NUMBER: 95177663 MEDLINE  
 DOCUMENT NUMBER: 95177663 PubMed ID: 7872795  
 TITLE: Characterization of flavin-containing monooxygenase 5 (FMO5) cloned from human and guinea pig: evidence that the unique catalytic properties of FMO5 are not confined to the rabbit ortholog.  
 AUTHOR: Overby L H; Buckpitt A R; Lawton M P; Atta-Asafo-Adjei E; Schulze J; Philpot R M  
 CORPORATE SOURCE: Molecular Pharmacology Section, National Institutes of Environmental Health Sciences, Research Triangle Park, North Carolina 27709.  
 SOURCE: ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, (1995 Feb 20) 317 (1) 275-84.  
 PUB. COUNTRY: Journal code: 6SK; 0372430. ISSN: 0003-9861.  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 OTHER SOURCE: GENBANK-L37080; GENBANK-L37081  
 ENTRY MONTH: 199503  
 ENTRY DATE: Entered STN: 19950407  
 Last Updated on STN: 19950407  
 Entered Medline: 19950329

AB Several full-length clones encoding the human and guinea pig orthologs of flavin-containing monooxygenase 5 (FMO5) have been isolated from libraries constructed with hepatic mRNA. The clones were detected by hybridization with the cDNA encoding FMO5 expressed in rabbit. The human and guinea pig cDNAs encode for proteins of 533 amino acids that contain putative pyrophosphate binding domains characteristic of mammalian FMOs. The sequences derived for the human and guinea pig FMO5 proteins are 87% identical and are 85 and 82% identical, respectively, to the sequence of rabbit FMO5. As is the case with other FMOs, FMO5 in human and guinea pig is encoded by multiple transcripts. Rabbit FMO5 expressed in *Escherichia coli* was purified and used to elicit antibodies in goat. These antibodies detected FMO5 in samples from livers of adult humans, rabbits, and guinea pigs and fetal livers of humans. The human and guinea pig forms of FMO5 were expressed in *E. coli* and characterized. Neither enzyme effectively catalyzed the metabolism of methimazole, a general FMO substrate; however, both were active with n-octylamine. The responses of the human FMO5 and guinea pig FMO5 to detergent, ions and elevated temperature are all similar to the responses described for rabbit FMO5. These results indicate that the unique properties of FMO5 from rabbit are species-independent and that this form of the flavin-containing monooxygenase is not readily classified as a drug-metabolizing enzyme.

L13 ANSWER 32 OF 42 MEDLINE DUPLICATE 23

ACCESSION NUMBER: 95236487 MEDLINE  
 DOCUMENT NUMBER: 95236487 PubMed ID: 7720103  
 TITLE: Role of hepatic flavin-containing monooxygenase 3 in drug and chemical metabolism in adult humans.  
 AUTHOR: Cashman J R; Park S B; Berkman C E; Cashman L E  
 CORPORATE SOURCE: Seattle Biomedical Research Institute, WA 98109, USA.  
 CONTRACT NUMBER: GM36426 (NIGMS)  
 SOURCE: CHEMICO-BIOLOGICAL INTERACTIONS, (1995 Apr 28) 96 (1) 33-46. Ref: 55  
 Journal code: CYV; 0227276. ISSN: 0009-2797.  
 PUB. COUNTRY: Ireland  
 Journal; Article; (JOURNAL ARTICLE)  
 General Review; (REVIEW)  
 (REVIEW, TUTORIAL)

## 09/583,310 Search Strategy/Results

LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199505  
ENTRY DATE: Entered STN: 19950605  
Last Updated on STN: 19950605  
Entered Medline: 19950525

AB In conjunction with asymmetric chemical syntheses and spectral, chiroptical, chromatographic and stereochemical correlation methods, we have developed procedures for the quantification of sulfoxide enantiomers and tertiary amine N-oxide diastereomer metabolites arising from the action of the adult human liver and other flavin-containing monooxygenases (FMOs). The parallel nature of the metabolic in vitro-in vivo studies and the use of chemical model oxidation systems allowed us to identify the FMO isoform involved. We investigated the enantioselective S-monooxygenation of cimetidine and the diastereoselective tertiary amine N-1'-oxygenation of (S)-nicotine as stereoselective functional probes of adult human liver FMO action. In both cases, the majority of evidence points to adult human liver FMO3 as the principal enzyme responsible for cimetidine S-oxygenation and (S)-nicotine N-1'-oxygenation in vitro and in vivo. The excellent agreement between the absolute configuration of the major cimetidine S-oxide and (S)-nicotine N-1'-oxide metabolites isolated from human urine and the major metabolite formed in the presence of adult human liver microsomes suggests that in vitro hepatic preparations may serve as a useful model for the in vivo condition. Further, that adult human liver cDNA-expressed FMO3 in Escherichia coli also gave the same absolute stereoselectivity (i.e. for (S)-nicotine N-1'-oxygenation) confirms the identity of the monooxygenase in vivo. Although we cannot rule out the involvement of minor contributions of cytochrome P-450 monooxygenases in cimetidine and (S)-nicotine oxidation, the majority of the data support the fact that cimetidine S-oxygenation and (S)-nicotine N-1'-oxygenation are stereoselective functional probes of adult human liver FMO3 activity. Finally, because the stereochemistry of the principal metabolite of cimetidine and (S)-nicotine in small experimental animals is distinct from that observed in humans, it is likely that species variation in predominant FMO isoforms exist and this may have important consequences for the choice of experimental animals in human preclinical drug design and development programs.

L13 ANSWER 33 OF 42 MEDLINE DUPLICATE 24  
ACCESSION NUMBER: 95236485 MEDLINE  
DOCUMENT NUMBER: 95236485 PubMed ID: 7720101  
TITLE: The molecular biology of the flavin-containing monooxygenases of man.  
AUTHOR: Phillips I R; Dolphin C T; Clair P; Hadley M R; Hutt A J; McCombie R R; Smith R L; Shephard E A  
CORPORATE SOURCE: Department of Biochemistry, Queen Mary and Westfield College, University of London, UK.  
SOURCE: CHEMICO-BIOLOGICAL INTERACTIONS, (1995 Apr 28) 96 (1) 17-32.  
Journal code: CYV; 0227276. ISSN: 0009-2797.  
PUB. COUNTRY: Ireland  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199505  
ENTRY DATE: Entered STN: 19950605  
Last Updated on STN: 19950605  
Entered Medline: 19950525

AB cDNA clones encoding five distinct members of the FMO family of man (FMOs 1, 2, 3, 4 and 5) were isolated by a combination of library screening and reverse transcription-polymerase chain reaction techniques. The deduced amino acid sequences of the human FMOs have 82-87% identity with their known orthologues in other mammal but only 51-57% similarity to each other. The hydropathy profiles of the proteins are very similar. From the calculated rate of evolution of FMOs (a 1% change in sequence per 6 million years) it would appear that individual members of the FMO gene family arose by duplication of a common ancestral gene some 250-300 million years ago. Each of the FMO genes was mapped by the polymerase chain reaction to the long arm of human chromosome 1. The localization of the FMO1 gene was further refined to 1q23-q25 by in situ hybridization of human metaphase chromosomes. RNase protection assays demonstrated that in man each FMO gene displays a distinct developmental and tissue-specific pattern of expression. In the adult, FMO1 is expressed in kidney but not in liver, whereas in the foetus its mRNA is abundant in

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both organs. FMO3 expression is essentially restricted to the liver in the adult and the mRNA is either absent, or present in low amounts, in foetal tissues. FMO4 is expressed more constitutively. Human FMO1 and FMO3 cDNAs were functionally expressed in prokaryotic and eukaryotic cells. FMO1 and FMO3, expressed in either system, displayed product stereoselectivity in their catalysis of the N-oxidation of the pro-chiral tertiary amines, N-ethyl-N-methylaniline (EMA) and pargyline. Both enzymes were stereoselective with respect to the production of the (-)-S-enantiomer of EMA N-oxide. But in the case of pargyline, the enzymes displayed opposite stereoselectivity, FMO1 producing solely the (+)-enantiomer and FMO3 predominantly the (-)-enantiomer of the N-oxide.

L13 ANSWER 34 OF 42 MEDLINE DUPLICATE 25  
 ACCESSION NUMBER: 95236486 MEDLINE  
 DOCUMENT NUMBER: 95236486 PubMed ID: 7720102  
 TITLE: Prochiral sulfides as in vitro probes for multiple forms of the flavin-containing monooxygenase.  
 AUTHOR: Rettie A E; Meier G P; Sadeque A J  
 CORPORATE SOURCE: Department of Medicinal Chemistry, University of Washington, Seattle 98195, USA.  
 SOURCE: CHEMICO-BIOLOGICAL INTERACTIONS, (1995 Apr 28) 96 (1) 3-15. Journal code: CYV; 0227276. ISSN: 0009-2797.  
 PUB. COUNTRY: Ireland  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199505  
 ENTRY DATE: Entered STN: 19950605  
 Last Updated on STN: 19950605  
 Entered Medline: 19950525

AB A homologous series of alkyl-substituted p-tolyl sulfides have been synthesized and evaluated as in vitro, isozyme-selective substrate probes for the microsomal flavin containing monooxygenases. Straight-chain and branched-chain alkyl homologs were metabolized to the corresponding (R)- and (S)-sulfoxides which were analyzed by chiral phase high-performance liquid chromatography. Initial studies demonstrated that the stereochemical composition of alkyl p-tolyl sulfoxides generated by FMO2, purified from rabbit lung, was a function of the degree of steric crowding about the prochiral center. In contrast, purified rabbit liver FMO1 formed the (R)-sulfoxide from the n-alkyl series of substrates in a highly stereoselective manner (> 90%). Similar results were obtained with these two rabbit cDNAs expressed in E. coli. In contrast to rabbit FMO1 and FMO2, a characteristic feature of catalysis by cDNA-expressed rabbit FMO3 was the lack of stereoselectivity observed for formation of methyl p-tolyl sulfoxide. Collectively, these data demonstrate that the stereochemical composition of sulfoxides generated from the n-alkyl series of sulfides is isozyme-dependent. Metabolism of methyl p-tolyl sulfide by detergent-solubilized hepatic microsomes from a wide variety of experimental animals yielded predominantly (R)-methyl p-tolyl sulfoxide, which, at least in rabbit liver, is indicative of catalysis dominated by FMO1. However, solubilized human and macaque liver preparations catalyzed this reaction in a relatively non-stereoselective manner. Macaque liver FMO was purified and the metabolite profile generated from the n-alkyl p-tolyl sulfides was found to be most similar to rabbit FMO3. Moreover, antibodies directed against macaque liver FMO selectively reacted with rabbit FMO3 and a microsomal protein expressed in adult human, but not fetal human liver, adult human kidney or adult human lung. Therefore, an FMO isoform expressed selectively in adult primate liver has catalytic and immunochemical properties consistent with its classification in the FMO3 family.

L13 ANSWER 35 OF 42 MEDLINE DUPLICATE 26  
 ACCESSION NUMBER: 94145088 MEDLINE  
 DOCUMENT NUMBER: 94145088 PubMed ID: 8311461  
 TITLE: A nomenclature for the mammalian flavin-containing monooxygenase gene family based on amino acid sequence identities.  
 AUTHOR: Lawton M P; Cashman J R; Cresteil T; Dolphin C T; Elfarra A A; Hines R N; Hodgson E; Kimura T; Ozols J; Phillips I R; +  
 CORPORATE SOURCE: National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709.  
 SOURCE: ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, (1994 Jan) 308 (1) 254-7. Journal code: 6SK; 0372430. ISSN: 0003-9861.

## 09/583,310 Search Strategy/Results

PUB. COUNTRY: United States  
 Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199403  
 ENTRY DATE: Entered STN: 19940330  
 Last Updated on STN: 19990129  
 Entered Medline: 19940315

AB A nomenclature based on comparisons of amino acid sequences is proposed for the members of the mammalian flavin-containing monooxygenase (FMO) gene family. This nomenclature is based on evidence of a single gene family composed of five genes. The percentage identities of the amino acid sequences of the five known forms of mammalian FMO are between 52 and 57% in rabbit and between 50 and 58% across species lines. The identities of all orthologs are greater than 82%. There is no evidence for multiple, highly related forms of the enzyme or for more than one mammalian FMO gene family. In the proposed system, the mammalian flavin-containing monooxygenase gene family is designated as "FMO" and the individual genes are distinguished by an Arabic numeral. The FMOs known as the "liver" and "lung" enzymes become FMO1 and FMO2, and the more recently described forms of the enzymes become FMO3, FMO4, and FMO5. Human FMO gene designations, FMO1 and FMO3, remain unchanged, but the gene designated FMO2 becomes FMO4. Following convention, the genes and cDNA designations will be italicized and the mRNA and protein designations will be nonitalicized. The purpose of the proposed nomenclature is to provide for the unambiguous identification of orthologous forms of mammalian FMOs, regardless of the species or tissue in question. The proposed classification considers only members of the mammalian flavin-containing monooxygenase gene family and has no bearing on the generally accepted definition of a multisubstrate flavin-containing monooxygenase.

L13 ANSWER 36 OF 42 MEDLINE DUPLICATE 27  
 ACCESSION NUMBER: 93252844 MEDLINE  
 DOCUMENT NUMBER: 93252844 PubMed ID: 8486656  
 TITLE: Cloning, sequencing, distribution, and expression in Escherichia coli of flavin-containing monooxygenase 1C1. Evidence for a third gene subfamily in rabbits.  
 AUTHOR: Atta-Asafo-Adjei E; Lawton M P; Philpot R M  
 CORPORATE SOURCE: Laboratory of Cellular and Molecular Pharmacology, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709.  
 SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1993 May 5) 268 (13) 9681-9.  
 Journal code: HIV; 2985121R. ISSN: 0021-9258.  
 PUB. COUNTRY: United States  
 Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 OTHER SOURCE: GENBANK-L08449  
 ENTRY MONTH: 199306  
 ENTRY DATE: Entered STN: 19930618  
 Last Updated on STN: 19970203  
 Entered Medline: 19930604

AB Two full-length cDNA clones (2.2 kilobases) encoding a newly recognized form of mammalian flavin-containing monooxygenase (FMO) have been isolated from independent libraries constructed with mRNA from different rabbits. The cDNAs encode a polypeptide of 533 amino acids which contains two putative pyrophosphate binding domains and a hydrophobic carboxyl terminus characteristic of FMOs. This sequence is 52 and 57% identical to sequences of the rabbit "hepatic" and "pulmonary" FMOs, respectively, and 55% identical to the sequence of "liver form 2" published recently by Ozols (Ozols, J. (1991) Arch. Biochem. Biophys. 290, 103-115). cDNA for the new FMO (FMO 1C1) hybridizes with two species of mRNA, one of 2.6 kilobases and one of about 5.4 kilobases, from liver or kidney, but not lung. Guinea pig, hamster, rat, and mouse all express this form of FMO in liver, kidney, and lung. FMO 1C1 has been tentatively characterized following expression in Escherichia coli. It is inactive with methimazole as substrate but highly active with n-octylamine. The temperature lability, responses to ions and detergent, and pH optimum of FMO 1C1 are similar to values reported for hepatic FMO. Sequence comparisons and analysis of rabbit and human genomic DNA indicate that FMO 1C1, as well as the pulmonary and hepatic FMOs, comprise a single gene family made up of distinct gene subfamilies (A, B,C,D, ... N), each appearing to contain a single gene. A nomenclature, based on these interrelationships and following the same designations used for classifying cytochromes P-450, is proposed.

L13 ANSWER 37 OF 42 MEDLINE DUPLICATE 28  
ACCESSION NUMBER: 94162508 MEDLINE  
DOCUMENT NUMBER: 94162508 PubMed ID: 8117928  
TITLE: Stereoselective metabolism of (S)-(-)-nicotine in humans:  
formation of trans-(S)-(-)-nicotine N-1'-oxide.  
AUTHOR: Park S B; Jacob P 3rd; Benowitz N L; Cashman J R  
CORPORATE SOURCE: Department of Pharmaceutical Chemistry and Liver Center,  
School of Pharmacy, University of California, San Francisco  
94143-0446.  
CONTRACT NUMBER: NIDA DAO1696 (NIDA)  
NIDA DAO2277 (NIDA)  
NIDA GM36426 (NIGMS)  
SOURCE: CHEMICAL RESEARCH IN TOXICOLOGY, (1993 Nov-Dec) 6 (6)  
880-8.  
Journal code: ASX; 8807448. ISSN: 0893-228X.  
PUB. COUNTRY: United States  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199404  
ENTRY DATE: Entered STN: 19940412  
Last Updated on STN: 19970203  
Entered Medline: 19940404

AB The chemical synthesis and chromatographic separation of cis- and trans-(S)-nicotine N-1'-oxide diastereomers have allowed the development of methods for the quantification of (S)-nicotine N-1'-oxides during in vitro and in vivo metabolic studies. The metabolism of (S)-nicotine was investigated in the presence of microsomes, cDNA-expressed and highly purified flavin-containing monooxygenase (FMO) from pig liver, human liver, and rabbit lung. For comparison, the N-1'-oxidation of (S)-nicotine in the presence of the cytochrome P450 2B1 from rat liver, cytochrome P450 2B10 from mouse liver, and cytochrome P450 4A2 from rabbit lung was examined. The ratio of trans:cis (S)-nicotine N-1'-oxide formation for pig liver FMO1 (form 1) was 57:43. In contrast, cDNA -expressed adult human liver FMO3 (form 3) and rabbit lung FMO2 formed solely trans-(S)-nicotine N-1'-oxide. Of the cytochrome P450 enzymes examined, formation of (S)-nicotine N-1'-oxide occurred with a mean trans:cis ratio of 82:18. The stereoselectivity of (S)-nicotine N-1'-oxide formation was investigated by examining the urine of 13 healthy male smokers studied on a protocol which included free-smoking, intravenous infusion of (S)-nicotine-d2 and dermal patch administration of (S)-nicotine-d0. During cigarette smoking or administration of intravenous or transdermal (S)-nicotine, only the trans diastereomer of (S)-nicotine N-1'-oxide was observed in the urine. That the trans-(S)-nicotine N-1'-oxide metabolite was not appreciably reduced or oxidized further was investigated with infusion studies of (S)-nicotine-d2N-1'-oxide. (ABSTRACT TRUNCATED AT 250 WORDS)

L13 ANSWER 38 OF 42 MEDLINE DUPLICATE 29  
ACCESSION NUMBER: 94162497 MEDLINE  
DOCUMENT NUMBER: 94162497 PubMed ID: 8117918  
TITLE: Regio- and stereoselective oxygenations by adult human liver flavin-containing monooxygenase 3. Comparison with forms 1 and 2.  
AUTHOR: Lomri N; Yang Z; Cashman J R  
CORPORATE SOURCE: Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco 94143-0446.  
CONTRACT NUMBER: GM 36426 (NIGMS)  
SOURCE: CHEMICAL RESEARCH IN TOXICOLOGY, (1993 Nov-Dec) 6 (6)  
800-7.  
Journal code: ASX; 8807448. ISSN: 0893-228X.  
PUB. COUNTRY: United States  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199404  
ENTRY DATE: Entered STN: 19940412  
Last Updated on STN: 19970203  
Entered Medline: 19940404

AB The cDNA for the adult human liver flavin-containing monooxygenase (form 3) (FMO3) was cloned, sequenced, and expressed in Escherichia coli. The cDNA-expressed FMO3 was used to investigate the regio- and stereoselective N- and S-oxygenation of a number of tertiary amines and sulfides, respectively. For comparison, the N- and S-oxygenation of the same chemicals and drugs were examined with adult human liver microsomes from a

normal healthy female donor and FMO1 from pig liver and FMO2 from rabbit lung. Both cDNA-expressed FMO3 and adult human liver microsomes N-oxygenated trifluoperazine or 10-(N,N-dimethylaminoalkyl)-phenothiazines with similar substrate specificities. The substrate specificity for FMO3 differed, however, from that of pig liver FMO1. Nucleophilic sulfur-containing compounds [i.e., thiobenzamide, (4-bromophenyl)-1,3-oxathiolane, and 2-methyl-1,3-benzodithiole] were efficiently S-oxygenated by cDNA-expressed FMO3 and adult human liver microsomes. Stereoselective S-oxygenation of (+)- and (-)-(4-bromophenyl)-1,3-oxathiolane and 2-methyl-1,3-benzodithiole was therefore investigated. In general, the stereoselectivity observed for S-oxygenation in the presence of FMO3 was similar to that observed in the presence of adult human liver microsomes. In most cases examined, however, the stereoselectivity for S-oxygenation was quite distinct from that observed for pig liver FMO1. We conclude that FMO3 is the major form of FMO active in adult human liver. Because the stereoselectivity for X-oxygenation and the substrate specificity for tertiary amine N-oxygenation by cDNA-expressed FMO3 are distinct from those of pig liver FMO1, we conclude that the binding channel for each isoform is quite different. (ABSTRACT TRUNCATED AT 250 WORDS)

L13 ANSWER 39 OF 42 MEDLINE DUPLICATE 30  
 ACCESSION NUMBER: 93385451 MEDLINE  
 DOCUMENT NUMBER: 93385451 PubMed ID: 8374037  
 TITLE: Expression in Escherichia coli of the flavin-containing monooxygenase D (form II) from adult human liver: determination of a distinct tertiary amine substrate specificity.  
 AUTHOR: Lomri N; Yang Z; Cashman J R  
 CORPORATE SOURCE: Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco 94143-0446.  
 CONTRACT NUMBER: TT 016614  
 SOURCE: CHEMICAL RESEARCH IN TOXICOLOGY, (1993 Jul-Aug) 6 (4) 425-9.  
 Journal code: A5X; 8807448. ISSN: 0893-228X.  
 PUB. COUNTRY: United States  
 Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199310  
 ENTRY DATE: Entered STN: 19931105  
 Last Updated on STN: 19970203  
 Entered Medline: 19931018

AB The cDNA for a major component of the family of flavin-containing monooxygenases (FMOs) present in adult human liver (i.e., HLFMO-D) has been cloned and expressed in a prokaryotic system. Escherichia coli strain NM522 was transformed with pTrcHLFMO-D, and the HLFMO-D cDNA was expressed under the control of the Trc promoter. A variety of tertiary amine substrates [i.e., chlorpromazine and 10-[(N,N-dimethylamino)alkyl]-2-(trifluoromethyl)phenothiazines] were efficiently oxygenated by HLFMO-D cDNA expressed in E. coli or by adult human liver microsomes. Approximate dimensions of the substrate binding channel for both adult human liver microsomal FMO and cDNA-expressed HLFMO-D were apparent from an examination of the N-oxygenation of a series of 10-[(N,N-dimethylamino)alkyl]-2-(trifluoromethyl)phenothiazines. The substrate regioselectivity studies suggest that adult human liver FMO form D possesses a distinct substrate specificity compared with form A FMO from animal hepatic sources. It is likely that the substrate specificity observed for cDNA-expressed adult human liver FMO-D may have consequences for the metabolism and distribution of tertiary amines and phosphorus- and sulfur-containing drugs in humans and may provide insight into the physiologic substrate(s) for adult human liver FMO.

L13 ANSWER 40 OF 42 MEDLINE DUPLICATE 31  
 ACCESSION NUMBER: 93277949 MEDLINE  
 DOCUMENT NUMBER: 93277949 PubMed ID: 8504165  
 TITLE: Rat liver flavin-containing monooxygenase (FMO): cDNA cloning and expression in yeast.  
 AUTHOR: Itoh K; Kimura T; Yokoi T; Itoh S; Kamataki T  
 CORPORATE SOURCE: Division of Drug Metabolism, Faculty of Pharmaceutical Sciences, Hokkaido University, Japan.  
 SOURCE: BIOCHIMICA ET BIOPHYSICA ACTA, (1993 May 28) 1173 (2) 165-71.



09/583,310 Search Strategy/Results

JOURNAL CODE: A0W; 0217513. ISSN: 0006-3002.  
 PUB. COUNTRY: Netherlands  
 JOURNAL; ARTICLE; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 OTHER SOURCE: GENBANK-D13124; GENBANK-D13127; GENBANK-D13132;  
 GENBANK-D13133; GENBANK-D13134; GENBANK-D13135;  
 GENBANK-D13136; GENBANK-D13137; GENBANK-L08068;  
 GENBANK-M84719  
 ENTRY MONTH: 199307  
 ENTRY DATE: Entered STN: 19930716  
 Last Updated on STN: 19950206  
 Entered Medline: 19930702

AB A rat liver cDNA clone, RFM01, coding for a  
 flavin-containing monooxygenase (FMO) was isolated. This cDNA  
 clone encoded a protein of 532 amino acids. The deduced amino acid  
 sequence was 84, 82 and 82% identical to those of the pig, human  
 (Form I) and rabbit (Form I) liver FMOs, while it was  
 only 52, 50, 54, 56 and 54% identical to the human (Form II),  
 human (Form 2) and rabbit liver FMOs (Form 2)  
 and rabbit and guinea pig lung FMOs. RNA blot analysis  
 showed that rat liver FMO was also expressed in lung and kidney  
 and to a lesser extent in the heart and brain. An expression plasmid,  
 pAMFMO, was constructed and the FMO protein expressed in yeast (AH22).  
 This FMO protein catalyzed thiobenzamide S-oxidation, and NADPH oxidation  
 associated with the S- or N-oxidation of thiourea, N,N-dimethylaniline,  
 trimethylamine, imipramine, chlorpromazine, N,N-dimethylhydrazine,  
 thioacetamide as substrates. The S-oxidation activities of thiobenzamide  
 and thiourea were enhanced by n-octylamine, a known enhancer of FMO, and  
 inhibited by alpha-naphthylthiourea, a known inhibitor of FMO. This is the  
 first report in which FMO with catalytic activities was stably expressed.

L13 ANSWER 41 OF 42 MEDLINE DUPLICATE 32  
 ACCESSION NUMBER: 92179247 MEDLINE  
 DOCUMENT NUMBER: 92179247 PubMed ID: 1542660  
 TITLE: Molecular cloning of the flavin-containing monooxygenase  
 (form II) cDNA from adult human  
 liver.  
 COMMENT: Erratum in: Proc Natl Acad Sci U S A 1995 Oct  
 10;92(21):9910  
 AUTHOR: Lomri N; Gu Q; Cashman J R  
 CORPORATE SOURCE: Department of Pharmaceutical Chemistry, School of Pharmacy,  
 University of California, San Francisco 94143-0446.  
 CONTRACT NUMBER: GM 36426 (NIGMS)  
 SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE  
 UNITED STATES OF AMERICA, (1992 Mar 1) 89 (5) 1685-9.  
 JOURNAL CODE: PV3; 7505876. ISSN: 0027-8424.  
 PUB. COUNTRY: United States  
 JOURNAL; ARTICLE; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 OTHER SOURCE: GENBANK-M83772  
 ENTRY MONTH: 199204  
 ENTRY DATE: Entered STN: 19920424  
 Last Updated on STN: 19960719  
 Entered Medline: 19920407

AB Complementary DNA (cDNA) clones encoding the adult  
 human liver flavin-containing monooxygenase (FMO;  
 dimethylaniline N-oxidase, EC 1.14.13.8) were isolated from lambda gt10  
 and lambda gt11 libraries. The cDNA libraries were screened with  
 three synthetic 36-mer oligonucleotide probes derived from the  
 nucleic acid sequence of the pig liver FMO cDNA  
 . The deduced amino acid sequence for the adult human  
 liver FMO was quite distinct from the pig liver FMO, and  
 adult human liver FMO was designated form II (HLFMO  
 II). The full-length cDNA sequence of HLFMO II [2119 base pairs  
 (bp)] had an open reading frame of 1599 nucleotides, which encoded a  
 533-amino acid protein of Mr 59,179, a 5'-noncoding region of 136  
 nucleotides and a 3'-noncoding region of 369 nucleotides excluding the  
 poly(A) tail. The deduced amino acid sequence of HLFMO II had 80%  
 similarity with the rabbit liver FMO II but only a 52%, 55%, and  
 53% amino acid similarity with the rabbit liver (form I), the  
 pig liver (form I), and fetal human liver  
 (form I) FMOs, respectively. RNA analysis of adult  
 human liver RNA showed that there was one  
 HLFMO II mRNA species. Analysis of genomic DNA  
 indicated that HLFMO II was the product of a single gene. These results  
 indicated that the deduced amino acid sequence for HLFMO II contained

highly conserved residues and suggested that FMO enzymes were closely related and, undoubtedly, derived from the same ancestral gene.

L13 ANSWER 42 OF 42 MEDLINE DUPLICATE 33

ACCESSION NUMBER: 93038564 MEDLINE

DOCUMENT NUMBER: 93038564 PubMed ID: 1417778

TITLE: Cloning, primary sequence and chromosomal localization of human FMO2, a new member of the flavin-containing mono-oxygenase family.

AUTHOR: Dolphin C T; Shephard E A; Povey S; Smith R L; Phillips I R

CORPORATE SOURCE: Department of Biochemistry, Queen Mary & Westfield College, University of London, U.K.

SOURCE: BIOCHEMICAL JOURNAL, (1992 Oct 1) 287 ( Pt 1) 261-7.  
Journal code: 9YO; 2984726R. ISSN: 0264-6021.

PUB. COUNTRY: ENGLAND: United Kingdom  
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

OTHER SOURCE: GENBANK-M86481; GENBANK-X65728; GENBANK-X65729;  
GENBANK-X65730; GENBANK-X65731; GENBANK-X65732;  
GENBANK-X65733; GENBANK-X65734; GENBANK-X66140;  
GENBANK-Z11737

ENTRY MONTH: 199211

ENTRY DATE: Entered STN: 19930122  
Last Updated on STN: 19930122  
Entered Medline: 19921110

AB We have previously reported the cloning of cDNAs for a flavin-containing mono-oxygenase (FMO) of man, designated FMO1 [Dolphin, Shephard, Povey, Palmer, Ziegler, Ayesh, Smith & Phillips (1991) J. Biol. Chem. 266, 12379-12385], that is the orthologue of pig and rabbit hepatic FMOs. We now describe the isolation and characterization of cDNA clones for a second human FMO, which we have designated FMO2. The polypeptide encoded by the cDNAs is 558 amino acid residues long, has a calculated M(r) of 63337, and contains putative FAD- and NADP-binding sites that align exactly with those described in other mammalian FMOs. Human FMO2 has 51-53% primary sequence identity with human FMO1, rabbit pulmonary FMO and rabbit liver FMO form 2, and thus represents a fourth, distinct, member of the mammalian FMO family. The corresponding mRNA is present in low abundance in adult human liver. Southern blot hybridization with single-exon probes demonstrated that human FMO2 and FMO1 are the products of single genes. The gene encoding FMO2 (designated FMO2) was mapped, by the polymerase chain reaction, to human chromosome 1, the same chromosome on which FMO1 is located.

QUESTED DATA FROM 3 ANSWERS - CONTINUE? Y/(N):y

L5 ANSWER 1 OF 3 MEDLINE DUPLICATE 1  
 ACCESSION NUMBER: 2001165255 MEDLINE  
 DOCUMENT NUMBER: 21163847 PubMed ID: 11266081  
 TITLE: A novel deletion in the flavin-containing monooxygenase gene (FMO3) in a Greek patient with trimethylaminuria.  
 AUTHOR: Forrest S M; Knight M; Akerman B R; Cashman J R; Treacy E P  
 CORPORATE SOURCE: Murdoch Children's Research Institute, Royal Children's Hospital, Parkville, Victoria, Australia.. forrest@cryptic.rch.unimelb.edu.au  
 CONTRACT NUMBER: GM36426 (NIGMS)  
 SOURCE: PHARMACOGENETICS, (2001 Mar) 11 (2) 169-74. Journal code: BRT; 9211735. ISSN: 0960-314X.  
 PUB. COUNTRY: England: United Kingdom  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 200106  
 ENTRY DATE: Entered STN: 20010618  
 Last Updated on STN: 20010618  
 Entered Medline: 20010614

AB Mutations of the flavin-containing monooxygenase type 3 gene (FMO3) that encode the major functional form present in adult human liver, have been shown to cause trimethylaminuria. We now report a novel homozygous deletion of exons 1 and 2 in an Australian of Greek ancestry with TMAuria, the first report of a deletion causative of trimethylaminuria. The deletion occurs 328 bp upstream from exon 1. The 3'-end of the deletion occurs in intron 2, 10013 base pairs downstream from the end of exon 2. The deletion is 12226 bp long. For the proband homozygous for the human FMO3 gene deletion, it is predicted that in addition to loss of monooxygenase function for human FMO3 substrates, such as TMA and other amines, the proband will exhibit decreased tolerance of biogenic amines, both medicinal and those found in foods.

L5 ANSWER 2 OF 3 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.  
 ACCESSION NUMBER: 95263022 EMBASE  
 DOCUMENT NUMBER: 1995263022  
 TITLE: Sequences promoting the transcription of the human XA gene overlapping P450c21A correctly predict the presence of a novel, adrenal-specific, truncated form of tenascin-X.  
 AUTHOR: Meng Kian Tee; Thomson A.A.; Bristow J.; Miller W.L.  
 CORPORATE SOURCE: Department of Pediatrics, Bldg. MR-IV, University of California, San Francisco, CA 94143-0978, United States  
 SOURCE: Genomics, (1995) 28/2 (171-178). ISSN: 0888-7543 CODEN: GNMCEP  
 COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article  
 FILE SEGMENT: 022 Human Genetics  
 029 Clinical Biochemistry  
 LANGUAGE: English  
 SUMMARY LANGUAGE: English

AB A compact region in the human class III major histocompatibility locus contains the human genes for the fourth component of human complement (C4) and steroid 21-hydroxylase (P450c21) in one transcriptional orientation, while the gene for the extracellular matrix protein tenascin-X (TN-X) overlaps the last exon of P450c21 on the opposite strand of DNA in the opposite transcriptional orientation. This complex locus is duplicated into A and B loci, so that the organization is 5'-C4A-21A-XA-C4B-21B-XB-3'. Although this duplication event truncated the 65-kb X(B) gene to a 4.5-kb XA gene, the XA gene is transcriptionally active in the adrenal cortex. To examine the basis of the tissue-specific expression of XA and C4B, we cloned the 1763-bp region that lies between the cap sites for XA and C4B and analyzed its promoter activity in both the XA and the C4 orientations. Powerful, liver-specific sequences lie within the first 75 to 138 bp from the C4B cap site, and weaker elements lie within 128 bp of the XA cap site that function in both liver and adrenal cells. Because these 128 bp upstream from the XA cap site are perfectly preserved in the XB gene encoding TN-X, we sought to determine whether a transcript similar to XA arises within the XB gene. RNase protection assays, cDNA cloning, and RT/PCR show that adrenal cells contain a novel transcript, termed short XB (XB-S), which has the same open reading frame as TN-X. Cell-free translation and immunoblotting show that this transcript encodes a novel 74-kDa XB-S protein that is identical to the carboxy-terminal 673 residues of TN-X. Because this protein consists solely of fibronectin type III repeats and a

fibrinogen-like domain, it appears to correspond to an evolutionary precursor of the tenascin family of extracellular matrix proteins.

L5 ANSWER 3 OF 3 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.  
 ACCESSION NUMBER: 92104186 EMBASE  
 DOCUMENT NUMBER: 1992104186  
 TITLE: Interleukin-1, platelet derived growth factor, free radicals and monocyte aryl hydrocarbon hydroxylase activity in liver disease. Role of cell communication.  
 AUTHOR: Peterson T.C.  
 CORPORATE SOURCE: Clinical Research Centre, Dalhousie University, Halifax, NS B3H 4H7, Canada  
 SOURCE: Biochemical Pharmacology, (1992) 43/5 (1163-1166). ISSN: 0006-2952 CODEN: BCPA6  
 COUNTRY: United Kingdom  
 DOCUMENT TYPE: Journal; Article  
 FILE SEGMENT: 005 General Pathology and Pathological Anatomy  
 026 Immunology, Serology and Transplantation  
 029 Clinical Biochemistry  
 048 Gastroenterology  
 037 Drug Literature Index  
 LANGUAGE: English  
 SUMMARY LANGUAGE: English

AB Monocytes were isolated from blood of human origin and cultured in supplemented leibovitz (L-15) medium for 24 hr. The medium was then decanted and filtered, and all subsequent tests were done on monocyte conditioned medium (MCM). The monocytes of patients with liver disease spontaneously secrete temperature-sensitive arylhydrocarbon hydroxylase (AHH) inhibitory factors detectable in the MCM. Anti-interleukin-1 antibody (IL-1Ab) reduced the AHH inhibitory activity of the MCM, suggesting that part of the AHH inhibitory activity was due to interleukin-1 (IL-1). Platelet derived growth factor did not affect AHH activity. Interleukin-1.beta. was detectable in MCM but did not differ significantly between patients and normal volunteers. A time course experiment indicated that interleukin-1.beta. inhibited hepatocyte AHH activity after only 2 hr of incubation. Catalase partially blocked the AHH inhibitory activity of MCM suggesting that activated oxygen intermediates are partially involved in the AHH inhibitory activity of the MCM. Simultaneous incubation of interleukin-1.beta. and catalase did not prevent or augment the inhibitory action of IL-1 on AHH activity. IL-1 stimulates collagen synthesis and elevates serum procollagen type 3 peptide (P-III-P). Results indicated that serum P-III-P was elevated in blood sources producing temperature-sensitive AHH inhibitory factor.

=> d 1- ibib abs

YOU HAVE REQUESTED DATA FROM 20 ANSWERS - CONTINUE? Y/(N):y

L15 ANSWER 1 OF 20 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2001:250568 CAPLUS

DOCUMENT NUMBER: 135:302281

TITLE: A novel deletion in the flavin-containing  
monooxygenase gene (FMO3) in a Greek patient  
with trimethylaminuria

AUTHOR(S): Forrest, Susan M.; Knight, Melanie; Akerman, Beverley  
R.; Cashman, John R.; Treacy, Eileen P.

CORPORATE SOURCE: Murdoch Children's Research Institute, Department of  
Paediatrics, Royal Children's Hospital, University of  
Melbourne, Parkville, 3052, Australia

SOURCE: Pharmacogenetics (2001), 11(2), 169-174

CODEN: PHMCEE; ISSN: 0960-314X

PUBLISHER: Lippincott Williams & Wilkins

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Mutations of the flavin-contg. monooxygenase type 3 gene (FMO3)  
that encode the major functional form present in adult human  
liver, have been shown to cause trimethylaminuria. We now report  
a novel homozygous deletion of exons 1 and 2 in an Australian of Greek  
ancestry with TMAuria, the first report of a deletion causative of  
trimethylaminuria. The deletion occurs 328 bp upstream from exon 1. The  
3'-end of the deletion occurs in intron 2, 10013 base pairs downstream  
from the end of exon 2. The deletion is 12226 bp long. For the proband  
homozygous for the human FMO3 gene deletion, it is predicted  
that in addn. to loss of monooxygenase function for  
human FMO3 substrates, such as TMA and other amines, the proband  
will exhibit decreased tolerance of biogenic amines, both medicinal and  
those found in foods.

REFERENCE COUNT: 21 THERE ARE 21 CITED REFERENCES AVAILABLE FOR THIS  
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 2 OF 20 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1997:645652 CAPLUS

DOCUMENT NUMBER: 127:315640

TITLE: N-oxygenation of phenethylamine to the trans-oxime by  
adult human liver  
flavin-containing monooxygenase and  
retroreduction of phenethylamine hydroxylamine by  
human liver microsomes

AUTHOR(S): Lin, Jing; Cashman, John R.

CORPORATE SOURCE: Seattle Biomedical Research Institute, Seattle, WA,  
USA

SOURCE: J. Pharmacol. Exp. Ther. (1997), 282(3), 1269-1279

CODEN: JPETAB; ISSN: 0022-3565

PUBLISHER: Williams & Wilkins

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The biogenic amine phenethylamine has been shown to be N-oxygenated by  
human flavin-contg. monooxygenase (FMO) (form 3) and  
human liver microsomes and, to a much lesser extent,  
N-oxygenated by porcine liver FMO1 and porcine liver  
microsomes but not by rabbit FMO2. Adult human liver  
microsomes catalyze the NADPH-dependent N-oxygenation of phenethylamine to  
the corresponding trans-oxime through the intermediacy of phenethyl  
hydroxylamine. In addn. to trans-oxime formation, phenethyl hydroxylamine  
is retroreduced to phenethylamine in the presence of human or  
porcine liver microsomes. Studies on the biochem. mechanism of  
N-oxygenation suggested that trans-oxime formation was dependent on the  
human FMO (form 3) and that retroredn. was stimulated by  
superoxide and dependent on a cytochrome P 450 system. These conclusions  
are based on studies examg. the effects of incubation conditions on  
phenethylamine N-oxygenation and the effect of reactive oxygen species on  
phenethyl hydroxylamine retroredn., resp. The pharmacol. activity of  
synthetic phenethyl hydroxylamine and phenethyl oxime with a no. of  
biogenic amine receptors and transporters was examd. in vitro. In all  
cases examd., the affinity of phenethyl hydroxylamine and the  
corresponding oxime for a biogenic transporter or receptors was very poor.  
The results suggest that the biogenic amine phenethylamine is efficiently  
sequentially N-oxygenated in the presence of human liver  
microsomes or cDNA-expressed FMO (form 3) to phenethyl hydroxylamine and  
then to oximes that are pharmacol. inactive and serve to terminate biol.  
activity. N-oxygenation of phenethylamine to the corresponding  
trans-oxime is a detoxication process that abrogates pharmacol. activity.

L15 ANSWER 3 OF 20 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1997:545511 CAPLUS

DOCUMENT NUMBER: 127:231026

TITLE: Characterization of two human  
flavin-containing monooxygenase (form 3)  
enzymes expressed in Escherichia coli as maltose  
binding protein fusions

AUTHOR(S): Brunelle, Alan; Bi, Yi-An; Lin, Jing; Russell, Brett;  
Luy, Lisa; Berkman, Clifford; Cashman, John

CORPORATE SOURCE: Seattle Biomedical Research Institute, Seattle, WA,  
98109-1651, USA

SOURCE: Drug Metab. Dispos. (1997), 25(8), 1001-1007  
CODEN: DMDSAI; ISSN: 0090-9556

PUBLISHER: Williams &amp; Wilkins

DOCUMENT TYPE: Journal

LANGUAGE: English

AB To examine the possibility for drug metab. polymorphism, adult human flavin-contg. monooxygenases (form 3) (EC 1.14.13.8) that differ at one amino acid were expressed in Escherichia coli as maltose binding protein fusions. The cDNA that was first reported during the cloning of adult human flavin-contg. monooxygenase was designated the wild type lys158 enzyme. A second cDNA has been identified as a common polymorphism in some human populations and was designated the glul58 enzyme. The cDNA that encodes both enzymes was subcloned into a high yield protein fusion expression system, expressed, and the protein was partially purified by affinity chromatog. and characterized for enzyme activity with selective functional substrate probes. N- and S-oxygenation activity of both enzymes was detd. with 10-(N,N-dimethylaminopentyl)-2-(trifluoromethyl)phenothiazine and Me p-tolyl sulfide, resp. It was found that expression of both lys158 and glul58 enzymes of the human flavin-contg. monooxygenase form 3 as fusions with the maltose binding protein resulted in an enzyme that was sol. and greatly stabilized and had a reduced requirement for detergent during enzyme purifn. and during the assay for activity. Expression of the fusion proteins has allowed the prepn. of stable and highly active enzyme at greater purity than was readily possible in the past. With the exception of the stability and soly. characteristics, the phys. and chem. properties of lys158 and glul58 maltose binding fusion proteins of human flavin-contg. monooxygenase form 3 variants resembled that of flavin-contg. monooxygenase enzyme activity assocd. with human liver microsomes and enzyme isolated from a previous Escherichia coli expression system that lacked the protein fusion. Comparison of the catalytic activity of the two fusion proteins showed that while both forms were active, there were differences in their substrate specificities. Expression of the adult human flavin-contg. monooxygenase form 3 as a maltose binding protein has allowed considerable advances over the previously reported cDNA-expressed enzyme systems and may provide the basis for examg. the role of the flavin-contg. monooxygenase in human xenobiotic or drug metab.

L15 ANSWER 4 OF 20 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1997:476104 CAPLUS

DOCUMENT NUMBER: 127:133707

TITLE: Detoxication of Tyramine by the Flavin-Containing  
Monooxygenase: Stereoselective Formation of  
the Trans Oxime

AUTHOR(S): Lin, Jing; Cashman, John R.

CORPORATE SOURCE: Seattle Biomedical Research Institute, Seattle, WA,  
98109, USA

SOURCE: Chem. Res. Toxicol. (1997), 10(8), 842-852  
CODEN: CRTOEC; ISSN: 0893-228X

PUBLISHER: American Chemical Society

DOCUMENT TYPE: Journal

LANGUAGE: English

AB In the presence of pig or adult human liver microsomes, tyramine was metabolized to the corresponding trans oxime through the intermediacy of the hydroxylamine. The requisite intermediate, (4-hydroxyphenethyl)hydroxylamine, was retroreduced to tyramine or converted stereoselectively to the trans oxime in the presence of pig or adult human liver microsomes. Studies of the effect of metabolic inhibitors suggested that formation of the trans oxime and retroredn. of the hydroxylamine were largely dependent on NADPH and the flavin-contg. monooxygenase (FMO) and cytochrome P 450, resp. The conclusion that FMO was predominantly responsible for trans oxime formation in human liver microsomes was based on the effect of incubation conditions on tyramine N-oxygenation and the

observation that cDNA-expressed human FMO3 also N-oxygenated tyramine to give exclusively the trans oxime. The synthetic hydroxylamine and oxime metabolites of tyramine were examd. for affinity to human and animal dopamine and serotonin receptors and the human dopamine transporter. For all of the receptors and for the transporter examd., the avidity of the hydroxylamine and oximes was greater than 10  $\mu$ M and beyond the effective concn. for physiol. relevance. The results suggested that tyramine was sequentially N-oxygenated in the presence of pig and human liver microsomes and cDNA-expressed FMO3 to the hydroxylamine and then to the di-N-hydroxylamine that was spontaneously dehydrated to the trans oxime. This may be facilitated by FMO through a nondissociative substrate-enzyme interaction. Based on the biogenic amine receptor or transporter affinity for the hydroxylamine and oxime metabolites of tyramine, N-oxygenation of tyramine by pig or human liver FMO may represent a detoxication reaction that terminates the pharmacol. activity of tyramine.

L15 ANSWER 5 OF 20 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1996:619254 CAPLUS

DOCUMENT NUMBER: 126:27762

TITLE: N-Oxygenation of Primary Amines and Hydroxylamines and Retroreduction of Hydroxylamines by Adult Human Liver Microsomes and Adult Human Flavin-Containing Monooxygenase  
3

AUTHOR(S): Lin, Jing; Berkman, Clifford E.; Cashman, John R.

CORPORATE SOURCE: Seattle Biomedical Research Institute, Seattle, WA, 98108, USA

SOURCE: Chem. Res. Toxicol. (1996), 9(7), 1183-1193

CODEN: CRTOEC; ISSN: 0893-228X

PUBLISHER: American Chemical Society

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Adult human liver microsomes catalyze the NADPH-dependent N-oxygenation of 10-N-(8-aminooctyl)-2-(trifluoromethyl)phenothiazine to the corresponding oximes through the intermediacy of the hydroxylamine. In the presence of adult human liver microsomes, the primary amine is stereoselectively converted to the cis-oxime, but addn. of the alternative competitive substrate hydroxylamine hydrochloride apparently decreases the amt. of aliph. hydroxylamine retro-redn. because an increase in oxime formation was obsd. In the presence of hydroxylamine hydrochloride, however, the oxime product recovered was formed with very low stereoselectivity. Studies on the biochem. mechanism of oxime formation suggested that cis-oxime formation in the presence of adult human liver microsomes was largely dependent on the human flavin-contg. monooxygenase (form 3). This conclusion is based on the effects of incubation conditions on product formation when compared to results obsd. in the presence of cDNA-expressed human FMO3. The retroredn. of the intermediate hydroxylamine was dependent on NADPH but was not catalyzed by human flavin-contg. monooxygenase (form 3) or any one of seven prominent cytochromes P 450 that have been well-characterized in the human liver microsomes examd. The results suggest that aliph. primary amines are efficiently sequentially N-oxygenated in the presence of human liver microsomes to hydroxylamines and then to oximes mainly by the human flavin-contg. monooxygenase. Retroredn. of the intermediate hydroxylamine is apparently facilitated by a novel but as yet poorly characterized enzyme system that does not employ any of the currently known well-characterized cytochrome P 450 enzymes present in adult human liver microsomes.

L15 ANSWER 6 OF 20 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1996:34513 CAPLUS

DOCUMENT NUMBER: 124:169246

TITLE: Molecular cloning of the flavin-containing monooxygenase (form II) cDNA from adult human liver. [Erratum to document cited in CA118:186740]

AUTHOR(S): Lomri, Nouredine; Gu, Qimin; Cashman, John R.

CORPORATE SOURCE: School Pharm, University California, San Francisco, CA, 94143-0446, USA

SOURCE: Proceedings of the National Academy of Sciences of the United States of America (1995), 92(21), 9910

CODEN: PNASA6; ISSN: 0027-8424

PUBLISHER: National Academy of Sciences

DOCUMENT TYPE: Journal

09/583,310 Search Strategy/Results

LANGUAGE: English  
 AB The errors were not reflected in the abstr. or the index entries.

L15 ANSWER 7 OF 20 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1995:895749 CAPLUS

DOCUMENT NUMBER: 123:329208

TITLE: In vitro hepatic metabolism of ABT-418 in chimpanzee (Pan troglodytes): a unique pattern of microsomal flavin-containing monooxygenase-dependent stereoselective N'-oxidation

AUTHOR(S): Rodrigues, A. David; Kukulka, Michael J.; Ferrero, James L.; Cashman, John R.

CORPORATE SOURCE: Drug Metabolism Department, Abbott Laboratories, Abbott Park, IL, 60064-3500, USA

SOURCE: Drug Metab. Dispos. (1995), 23(10), 1143-52  
 CODEN: DMSAI; ISSN: 0090-9556

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Metab. of the cholinergic channel activator [N-methyl-3H]ABT-418 was studied using precision-cut tissue slices and microsomes (.-.cytosol) prep'd. from a single chimpanzee liver. In both cases, the products of C-oxidn. (lactam) and N'-oxidn. (cis > trans) were detected. In the presence of chimpanzee liver microsomes and cytosol, which had been characterized with respect to the levels of aldehyde oxidase (N1-methylnicotinamide oxidase), NADPH-dependent flavin-contg. monooxygenase (FMO; N,N-dimethylaniline N-oxidase), and various cytochrome P 450 (CYP)-dependent monooxygenase activities, ABT-418 lactam and N'-oxide formation was largely dependent on CYP/aldehyde oxidase and FMO, resp. The rank order of total (trans + cis) FMO-dependent N'-oxidn. in liver microsomes was dog > rat > rabbit > chimpanzee .gtoreq. cynomolgus monkey > human. It is concluded that the metabolic profile of ABT-418 in the chimpanzee is unique. First, the C-/N'-oxidn. ratio in liver slices (0.43) is similar to that of the rat and dog and dissimilar to that of the two other primate species studied; human and cynomolgus monkey (C-/N'-oxidn. ratio .gtoreq. 9.4). Second, the pattern of ABT-418 N'-oxidn. obsd.. with chimpanzee liver microsomes, and liver slices (trans:cis = 1:3), differs from that of rat, rabbit, and dog liver microsomes, rat and human kidney S-9 (trans .mchgt. cis), human liver microsomes (trans:cis .apprx. 1:1), and cynomolgus monkey (trans:cis .apprx. 2:1) liver microsomes. Lack of stereoselective N'-oxidn. by human FMO was confirmed with cDNA-expressed FMO3.

L15 ANSWER 8 OF 20 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1995:874565 CAPLUS

TITLE: Molecular cloning of the flavin-containing monooxygenase (form II) cDNA from adult human liver

AUTHOR(S): Lomri, N.; Gu, Q.; Cashman, J. R.

SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1995), 92(21), 9910  
 CODEN: PNASA6; ISSN: 0027-8424

DOCUMENT TYPE: Journal; Errata

LANGUAGE: English

AB Unavailable

L15 ANSWER 9 OF 20 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1995:758120 CAPLUS

DOCUMENT NUMBER: 123:163167

TITLE: In vitro-in vivo correlations of human (S)-nicotine metabolism

AUTHOR(S): Berkman, Clifford E.; Park, Sang B.; Wrighton, Steven A.; Cashman, John R.

CORPORATE SOURCE: Seattle Biomedical Research Institute, Seattle, WA, 98109, USA

SOURCE: Biochem. Pharmacol. (1995), 50(4), 565-70  
 CODEN: BCPA6; ISSN: 0006-2952

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The profile of (S)-nicotine metab. in human liver microsomes was exam'd. at concns. approaching in vivo conditions (10 .mu.M). At such concns., no (S)-nicotine N-1'-oxygenation was seen, and thus C-oxidn. to the (S)-nicotine .DELTA.1',5'-iminium ion was the sole product obsd. in the metabolic profile in the presence of the human liver microsomes. For simplicity of anal., the (S)-nicotine .DELTA.1',5'-iminium ion formed was converted to (S)-cotinine in the presence of exogenously added aldehyde oxidase. To explain the lack of (S)-nicotine N-1'-oxygenation at low (S)-nicotine concns.,



inhibition of flavin-contg. monooxygenase (FMO) activity by (S)-cotinine was examd. Although (S)-cotinine was obsd. to inhibit pig FMO1 ( $K_i = 675 \mu\text{M}$ ), partially purified cDNA-expressed adult human liver FMO3 was not inhibited by (S)-cotinine. The authors therefore concluded that the kinetic properties of the nicotine N'- and C-oxidases were responsible for the metabolic product profile obsd. Kinetic consts. were detd. for individual human liver microsomal preps. from low ( $10 \mu\text{M}$ ) and high ( $500 \mu\text{M}$ ) (S)-nicotine concns. by monitoring (S)-cotinine formation with HPLC. The mean  $K_{\text{mapp}}$  and  $V_{\text{max}}$  for formation of (S)-cotinine by the microsomes examd. were  $39.6 \mu\text{M}$  and  $444.3 \text{ pmol} \cdot \text{cntdot} \cdot \text{min}^{-1} \cdot (\text{mg protein})^{-1}$ , resp. The formation of (S)-cotinine was strongly dependent on the previous drug administration history of each subject, and among the highest rates for (S)-cotinine formation were those of the barbiturate-pretreated subjects. The rate of (S)-cotinine formation at low ( $10 \mu\text{M}$ ) concn. correlated well with immunoreactivity for cytochrome P 450 2A6 ( $r = 0.89$ ). In vitro-in vivo correlation of the results suggests that the low amt. of (S)-nicotine N-1'-oxygenation and the large amt. of (S)-cotinine formed in human smokers (i.e. 4 and 30% of a typical dose, resp.) are detd. primarily by the kinetic properties of the human monooxygenase enzyme systems. However, addnl. non-hepatic monooxygenase(s) contributes to (S)-nicotine metab.

L15 ANSWER 10 OF 20 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1995:495570 CAPLUS

DOCUMENT NUMBER: 122:281214

TITLE: Role of hepatic flavin-containing monooxygenase 3 in drug and chemical metabolism in adult humans

AUTHOR(S): Cashman, John R.; Park, Sang B.; Berkman, Clifford E.; Cashman, Lisa E

CORPORATE SOURCE: Seattle Biomedical Research Institute, 4 Nickerson Street, Suite 200, Seattle, WA, 98109, USA

SOURCE: Chem.-Biol. Interact. (1995), 96(1), 33-46  
CODEN: CBINA8; ISSN: 0009-2797

DOCUMENT TYPE: Journal; General Review

LANGUAGE: English

AB A review, with 55 refs. In conjunction with asym. chem. syntheses and spectral, chiroptical, chromatog. and stereochem. correlation methods, the authors have developed procedures for the quantification of sulfoxide enantiomers and tertiary amine N-oxide diastereomer metabolites arising from the action of the adult human liver and other flavin-contg. monooxygenases (FMOs). The parallel nature of the metabolic in vitro-in vivo studies and the use of chem. model oxidn. systems allowed the authors to identify the FMO isoform involved. The authors investigated the enantioselective S-monooxygenation of cimetidine and the diastereoselective tertiary amine N-1'-oxygenation of (S)-nicotine as stereoselective functional probes of adult human liver FMO action. In both cases, the majority of evidence points to adult human liver FMO3 as the principal enzyme responsible for cimetidine S-oxygenation and (S)-nicotine N-1'-oxygenation in vitro and in vivo. The excellent agreement between the abs. configuration of the major cimetidine S-oxide and (S)-nicotine N-1'-oxide metabolites isolated from human urine and the major metabolite formed in the presence of adult human liver microsomes suggests that in vitro hepatic preps. may serve as a useful model for the in vivo condition. Further, that adult human liver cDNA-expressed FMO3 in Escherichia coli also gave the same abs. stereoselectivity (i.e. for (S)-nicotine N-1'-oxygenation) confirms the identity of the monooxygenase in vivo. Although the authors cannot rule out the involvement of minor contributions of cytochrome P 450 monooxygenases in cimetidine and (S)-nicotine oxidn., the majority of the data support the fact that cimetidine S-oxygenation and (S)-nicotine N-1'-oxygenation are stereoselective functional probes of adult human liver FMO3 activity. Finally, because the stereochem. of the principal metabolite of cimetidine and (S)-nicotine in small exptl. animals is distinct from that obsd. in humans, it is likely that species variation in predominant FMO isoforms exist and this may have important consequences for the choice of exptl. animals in human preclin. drug design and development programs.

L15 ANSWER 11 OF 20 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1994:207839 CAPLUS

DOCUMENT NUMBER: 120:207839

TITLE: Stereo- and regioselective N- and S-oxidation of tertiary amines and sulfides in the presence of adult human liver microsomes. [Erratum to document cited in CA119(11):108339g]

09/583,310 Search Strategy/Results

AUTHOR(S): Cashman, John R.; Yang, Zicheng; Wang, Lihong; Wrighton, Steven A.  
 CORPORATE SOURCE: Sch. Pharm., Univ. California, San Francisco, CA, USA  
 SOURCE: Drug Metab. Dispos. (1993), 21(6), 1174  
 CODEN: DMDSAI; ISSN: 0090-9556  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English  
 AB The errors were not reflected in the abstr. or the index entries.

L15 ANSWER 12 OF 20 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1994:92 CAPLUS  
 DOCUMENT NUMBER: 120:92  
 TITLE: Comparison of human and rhesus monkey in vitro phase I and phase II hepatic drug metabolism activities

AUTHOR(S): Stevens, Jeffrey; Shipley, Lisa A.; Cashman, John R.; Vandenbranden, Mark; Wrighton, Steven A.  
 CORPORATE SOURCE: Dep. Drug Metab. Disposit., Eli Lilly and Co., Indianapolis, IN, 46285, USA  
 SOURCE: Drug Metab. Dispos. (1993), 21(5), 753-60  
 CODEN: DMDSAI; ISSN: 0090-9556

DOCUMENT TYPE: Journal  
 LANGUAGE: English

AB Twelve human and six rhesus monkey liver samples were analyzed in vitro for phase I metab. and phase II conjugation activity. Of the eight P 450-dependent activities measured, only N-nitrosodimethylamine N-demethylase activity was not significantly different between the two species. Coumarin 7-hydroxylase activity was greater in the human as compared with the rhesus monkey samples, whereas erythromycin N-demethylase, benzphetamine N-demethylase, pentoxifyresorufin O-dealkylase, ethoxycoumarin O-deethylase, and ethoxymresorufin O-deethylase activities were significantly greater in rhesus monkey microsomal samples. Cimetidine S-oxygenation and chlorpromazine N-oxygenation were 2.1- and 2.6-fold higher in rhesus monkey samples. Of the seven microsomal and cytosolic phase II activities measured, only 17.alpha.-ethynylestradiol glucuronidation was significantly higher in the human samples. The genetic polymorphism for isoniazid acetylation was evident only in the human samples, with activities varying 200-fold. This study shows that, although the rhesus monkey is often used by the pharmaceutical industry as a representative mammalian species for drug testing, the in vitro metabolic capabilities of the human and rhesus monkey drug metabolizing enzymes are different.

L15 ANSWER 13 OF 20 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1993:664287 CAPLUS  
 DOCUMENT NUMBER: 119:264287  
 TITLE: Regio- and stereoselective oxygenations by adult human liver flavin-containing monooxygenase 3. Comparison with forms 1 and 2

AUTHOR(S): Lomri, Nouredine; Yang, Zicheng; Cashman, John R.  
 CORPORATE SOURCE: Sch. Pharm., Univ. California, San Francisco, CA, 94143-0446, USA  
 SOURCE: Chem. Res. Toxicol. (1993), 6(6), 800-7  
 CODEN: CRTOEC; ISSN: 0893-228X

DOCUMENT TYPE: Journal  
 LANGUAGE: English

AB The cDNA for the adult human liver flavin-contg. monooxygenase (form 3) (FMO3) was cloned, sequenced, and expressed in Escherichia coli. The cDNA-expressed FMO3 was used to investigate the regio- and stereoselective N- and S-oxygenation of a no. of tertiary amines and sulfides, resp. For comparison, the N- and S-oxygenation of the same chems. and drugs were examd. with adult human liver microsomes from a normal healthy female donor and FMO1 from pig liver and FMO2 from rabbit lung. Both cDNA-expressed FMO3 and adult human liver microsomes N-oxygenated trifluoperazine or 10-(N,N-dimethylaminoalkyl)-phenothiazines with similar substrate specificities. The substrate specificity for FMO3 differed, however, from that of pig liver FMO1. Nucleophilic sulfur-contg. compds. [i.e., thiobenzamide, (4-bromophenyl)-1,3-oxathiolane, and 2-methyl-1,3-benzodithiole] were efficiently S-oxygenated by cDNA-expressed FMO3 and adult human liver microsomes. Stereoselective S-oxygenation of (+)- and (-)-(4-bromophenyl)-1,3-oxathiolane and 2-methyl-1,3-benzodithiole was therefore investigated. In general, the stereoselectivity obsd. for S-oxygenation in the presence of FMO3 was similar to that obsd. in the presence of adult human liver microsomes. In most

cases examd., however, the stereoselectivity for S-oxygenation was quite distinct from that obsd. for pig liver FMO1. The authors conclude that FMO3 is the major form of FMO active in adult human liver. Because the stereoselectivity for S-oxygenation and the substrate specificity for tertiary amine N-oxygenation by cDNA-expressed FMO3 are distinct from those of pig liver FMO1, the authors conclude that the binding channel for each isoform is quite different. Like FMO2 from rabbit lung, FMO3 apparently possesses a much smaller substrate binding channel than pig liver FMO1, and this undoubtedly has consequences for tertiary amine and sulfide metab. in humans.

L15 ANSWER 14 OF 20 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1993:661944 CAPLUS

DOCUMENT NUMBER: 119:261944

TITLE: Chemical, enzymic, and human enantioselective S-oxygenation of cimetidine

AUTHOR(S): Cashman, John R.; Park, Sang B.; Yang, Zi Chen; Washington, Carla B.; Gomez, Denise Y.; Giacomini, Kathleen M.; Brett, Claire M.

CORPORATE SOURCE: Dep. Pharm. Chem., Univ. California, San Francisco, CA, USA

SOURCE: Drug Metab. Dispos. (1993), 21(4), 587-97

CODEN: DMSDAI; ISSN: 0090-9556

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The S-oxygenation of cimetidine was investigated using achiral chem. and chiral chem. and enzymic S-oxygenation procedures. The products of the reactions were thoroughly characterized by spectral, chiroptical, chromatog., and stereochem. correlation methods. S-Oxygenation by the Kagan method or in the presence of pig liver microsomes or pig liver flavin-contg. monooxygenase (FMO) (form I) all gave essentially identical enantioselectivity: the av. enantiomeric excess was -13.4% and the stereopreference was for formation of (+)-cimetidine S-oxide in a ratio of (+)56.7%:(-)43.3%. The profile of immunoreactivity and the effect of metab. inhibitors on cimetidine S-oxide formation in the presence of pig liver microsomes were consistent with a role of FMO (form I) in enantioselective (+)-cimetidine S-oxide formation. Administration of cimetidine to seven healthy male volunteers provided pharmacokinetic parameters for cimetidine and cimetidine S-oxide that were typical of those for previously reported studies. The urinary cimetidine S-oxide was isolated and the stereopreference was for formation of (-)-cimetidine S-oxide in a ratio of (+)25.5%:(-)74.5%. In good agreement with the enantiomeric enrichment values obsd. for the adult human urinary metabolite, the relative configuration of cimetidine S-oxide formed in adult human liver microsomes was (+)-15.8%:(-)-84.2%. Because of the enantioselectivity and profile of immunoreactivity and the effect of metab. inhibitors on cimetidine S-oxygenation in adult human liver microsomes are consistent with a role of FMO (form II) in cimetidine S-oxide formation and because the enantioselectivity of cimetidine S-oxide obsd. in adult humans is similar, the authors conclude that in vivo, cimetidine is S-oxygenated principally by FMO (form II).

L15 ANSWER 15 OF 20 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1993:643495 CAPLUS

DOCUMENT NUMBER: 119:243495

TITLE: Stereoselective metabolism of (S)-(-)-nicotine in humans: Formation of trans-(S)-(-)-nicotine N-1'-oxide

AUTHOR(S): Park, Sang B.; Jacob, Peyton, III; Benowitz, Neal L.; Cashman, John R.

CORPORATE SOURCE: Sch. Pharm., Univ. California, San Francisco, CA, 94143-0446, USA

SOURCE: Chem. Res. Toxicol. (1993), 6(6), 880-8

CODEN: CRTOEC; ISSN: 0893-228X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The metab. of (S)-nicotine was investigated in the presence of microsomes, cDNA-expressed and highly purified flavin-contg. monooxygenase (FMO) from pig liver, human liver, and rabbit lung. For comparison, the N-1'-oxidn. of (S)-nicotine in the presence of the cytochrome P 450 2B1 from rat liver, cytochrome P 450 2B10 from mouse liver, and cytochrome P 450 4A2 from rabbit lung was examd. The ratio of trans:cis (S)-nicotine N-1'-oxide formation for pig liver FMO (form I) was 57:43. In contrast, cDNA-expressed adult human liver FMO (form 3) and rabbit lung FMO2 formed solely trans (S)-nicotine N-1'-oxide. Of the

cytochrome P 450 enzymes examd., formation of (S)-nicotine N-1'-oxide occurred with a mean trans:cis ratio of 82:18. The stereoselectivity of (S)-nicotine N-1'-oxide formation was investigated by examg. the urine of 13 healthy male smokers studied on a protocol which included free-smoking, i.v. infusion of (S)-nicotine-d2 and dermal patch administration of (S)-nicotine-d0. During cigarette smoking or administration of i.v. or transdermal (S)-nicotine, only the trans diastereomer of (S)-nicotine N-1'-oxide was obsd. in the urine. That the trans (S)-nicotine N-1'-oxide metabolite was not appreciably reduced or oxidized further was investigated with infusion studies of (S)-nicotine-d2 N-1'-oxide. The mean trans:cis (S)-nicotine N-1'-oxide ratio detd. from the metabolite isolated from the urine of humans after infusion of the N-1'-oxide was 60:40, which was essentially identical to that of the infusate. Previously, the authors have obsd. exclusive trans-(S)-nicotine N-1'-oxide formation in the presence of 14 different adult human liver microsome samples. As described herein, after administration of (S)-nicotine to 13 healthy adult smokers by 3 different routes of administration, the authors also obsd. only trans-(S)-nicotine N-1'-oxide formation. The excellent agreement between in vitro and in vivo results suggests that human (S)-nicotine N-1'-oxygenation is catalyzed predominantly by one monooxygenase. The majority of the data strongly suggests that the adult human liver flavin-contg. monooxygenase (form 3) is responsible for trans-(S)-nicotine N-1'-oxygenation, and the authors propose that formation of this metabolite is a selective functional marker for the enzyme.

L15 ANSWER 16 OF 20 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1993:508339 CAPLUS

DOCUMENT NUMBER: 119:108339

TITLE: Stereo- and regioselective N- and S-oxidation of tertiary amines and sulfides in the presence of adult human liver microsomes

AUTHOR(S): Cashman, John R.; Yang, Zicheng; Yang, Lihong; Wrighton, Steven A.

CORPORATE SOURCE: Sch. Pharm., Univ. California, San Francisco, CA, USA

SOURCE: Drug Metab. Dispos. (1993), 21(3), 492-501

CODEN: DMSDAI; ISSN: 0090-9556

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Adult human liver microsomes supplemented with NADPH catalyzed the regioselective N-oxygenation of the aliph. tertiary amine and S-oxidn. of the phenothiazine sulfur atom of several 10-(N,N-dimethylaminoalkyl)phenothiazines. In addn., (+)- and (-)-4-bromophenyl-1,3-oxathiolane were converted to the corresponding S-oxides in the presence of NADPH and adult human liver microsomes. The (+) and (-) enantiomers of 4-bromophenyl-1,3-oxathiolane were converted to the S-oxides with low and high stereoselectivity, resp. Studies on the biochem. mechanism for N-oxygenation of 10-(N,N-dimethylaminoalkyl)phenothiazines suggested that this reaction was catalyzed by the flavin-contg. monooxygenase (form II), although cytochrome P 450 2D6 may also have contributed to N-oxide formation. S-Oxidn. of chlorpromazine was catalyzed mainly by cytochrome P 450, including cytochromes P 450 2A6, 2C8, and 2D6. S-Oxygenation of (+)-4-bromophenyl-1,3-oxathiolane produced a mixt. of the cis- and trans diastereomers in a process probably dependent on both hepatic monooxygenase systems. (-)-4-Bromophenyl-1,3-oxathiolane was converted almost exclusively to the trans-S-oxide in a process likely dependent on the adult human liver flavin-contg. monooxygenase (form II). Development of regio- and stereochem. probes of adult human liver flavin-contg. monooxygenase (form II) and cytochromes P 450 activity may be useful for eventual in vitro-in vivo correlations, but may require approaches quite distinct from that currently used for animal monooxygenases.

L15 ANSWER 17 OF 20 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1993:466327 CAPLUS

DOCUMENT NUMBER: 119:66327

TITLE: Expression in Escherichia coli of the flavin-containing monooxygenase D (form II) from adult human liver: Determination of a distinct tertiary amine substrate specificity

AUTHOR(S): Lomri, Noureddine; Yang, Zicheng; Cashman, John R.

CORPORATE SOURCE: Sch. Pharm., Univ. California, San Francisco, CA, 94143-0446, USA

## 09/583,310 Search Strategy/Results

SOURCE: Chem. Res. Toxicol. (1993), 6(4), 425-9

CODEN: CRTOEC; ISSN: 0893-228X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The cDNA for a major component of the family of flavin-contg. monooxygenases (FMOs) present in adult human liver (i.e., HLFMO-D) has been cloned and expressed in a prokaryotic system. Escherichia coli strain NM522 was transformed with pTrcHLFMO-D, and the HLFMO-D cDNA was expressed under the control of the Trc promoter. A variety of tertiary amine substrates [i.e., chlorpromazine and 10-[(N,N-dimethylamino)alkyl]-2-(trifluoromethyl)phenothiazines] were efficiently oxygenated by HLFMO-D cDNA expressed in E. coli or by adult human liver microsomes. Approx. dimensions of the substrate binding channel for both adult human liver microsomal FMO and cDNA-expressed HLFMO-D were apparent from an examn. of the N-oxygenation of a series of 10-[(N,N-dimethylamino)alkyl]-2-(trifluoromethyl)phenothiazines. The substrate regioselectivity studies suggest that adult human liver FMO form D possesses a distinct substrate specificity compared with form A FMO from animal hepatic sources. It is likely that the substrate specificity obsd. for cDNA-expressed adult human liver FMO-D may have consequences for the metab. and distribution of tertiary amines and phosphorus- and sulfur-contg. drugs in humans and may provide insight into the physiol. substrate(s) for adult human liver FMO.

L15 ANSWER 18 OF 20 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1993:186740 CAPLUS

DOCUMENT NUMBER: 118:186740

TITLE: Molecular cloning of the flavin-containing monooxygenase (form II) cDNA from adult human liver

AUTHOR(S): Lomri, Nouredine; Gu, Qimin; Cashman, John R.

CORPORATE SOURCE: Sch. Pharm., Univ. California, San Francisco, CA, 94143-0446, USA

SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1992), 89(5), 1685-9

CODEN: PNASAA6; ISSN: 0027-8424

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Several cDNA clones encoding the adult human liver flavin-contg. monooxygenase (FMO; dimethylaniline N-oxidase, EC 1.14.13.8) were isolated from .lambda.gt10 and .lambda.gt11 libraries. The cDNA libraries were screened with 3 synthetic 36-mer oligonucleotide probes derived from the nucleic acid sequence of pig liver FMO cDNA. The deduced amino acid sequence for adult human liver FMO was quite distinct from pig liver FMO, and adult human liver FMO was designated form II (HLFMO II). The full-length cDNA sequence of HLFMO II [2119 base pairs (bp)] had an open reading frame of 1599 nucleotides, which encoded a 533-amino acid protein of Mr 59,179, a 5'-noncoding region of 136 nucleotides and a 3'-noncoding region of 369 nucleotides, excluding the poly(A) tail. The deduced amino acid sequence of HLFMO II had 80% similarity with the rabbit liver FMO II but only 52%, 55%, and 53% amino acid similarity with rabbit liver (form I), pig liver (form I), and fetal human liver (form I) FMOs, resp. RNA anal. of adult human liver RNA showed that there was one HLFMO II mRNA species. Anal. of genomic DNA indicated that HLFMO II was the product of a single gene. Thus, the deduced amino acid sequence for HLFMO II contained highly conserved residues and suggested that FMO enzymes were closely related and, undoubtedly, derived from the same ancestral gene.

L15 ANSWER 19 OF 20 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1992:545205 CAPLUS

DOCUMENT NUMBER: 117:145205

TITLE: Metabolism of nicotine by human liver microsomes: stereoselective formation of trans-nicotine N'-oxide

AUTHOR(S): Cashman, John R.; Park, Sang B.; Yang, Z. C.; Wrighton, Steven A.; Jacob, Peyton, III; Benowitz, Neal L.

CORPORATE SOURCE: Sch. Pharm., Univ. California, San Francisco, CA, 94143-0446, USA

SOURCE: Chem. Res. Toxicol. (1992), 5(5), 639-46

CODEN: CRTOEC; ISSN: 0893-228X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Liver microsomes from humans catalyze the NADPH-dependent oxidn. of (S)-nicotine. The principal product is the 5'-carbon atom oxidn. product, nicotine .DELTA.1',5'-iminium ion, which is efficiently converted

to the .gamma.-lactam deriv. cotinine in the presence of aldehyde oxidase. Another major product is nicotine N'-oxide. In contrast to previous reports, describing in vitro or in vivo studies, formation of only trans-nicotine N'-oxide was obsd. Demethylation of nicotine was not obsd. Studies on the biochem. mechanism of nicotine 5-carbon atom oxidn. strongly implicate one major cytochrome P 450 isoenzyme (i.e., P 450 2A6) as largely responsible for .DELTA.1',5'-iminium ion formation. Stereoselective formation of trans-nicotine N'-oxide may be catalyzed in large part by the flavin-contg. **monooxygenase** (form II). These conclusions are based on the effects of alternative substrates for the flavin-contg. **monooxygenase**, heat inactivation studies, immunoblot studies, and selective substrates for cytochromes P 450. The results suggest that (S)-nicotine trans N'-oxygenation and .DELTA.1',5'-iminium ion formation may be selective probes of **human liver flavin-contg. monooxygenase form II** and cytochrome P 450 2A6 activities, resp., useful for in vivo phenotyping of humans.

L15 ANSWER 20 OF 20 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1986:179654 CAPLUS

DOCUMENT NUMBER: 104:179654

TITLE: Contribution of N-oxygenation to the metabolism of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) by various liver preparations

AUTHOR(S): Cashman, John R.; Ziegler, D. M.

CORPORATE SOURCE: Sch. Pharm., Univ. California, San Francisco, CA, 94143, USA

SOURCE: Mol. Pharmacol. (1986), 29(2), 163-7

CODEN: MOPMA3; ISSN: 0026-895X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB **Liver** microsomes from uninduced mice and rats catalyzed NADPH- and O-dependent N-oxygenation of the neurotoxin (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [28289-54-5]. The N-oxide [95969-40-7] is the principal product and accounts for 95-96% of the total MPTP metabolized by microsomes. Demethylation of MPTP is detectable but the rate of nor-MPTP [10338-69-9] formation is never >4-6% of the rate of N-oxygenation. Studies on the biochem. mechanisms for N-oxygenation of MPTP suggest that this reaction is catalyzed exclusively by the flavin **monooxygenase** [37256-73-8]. This conclusion is based on the effects of selective cytochrome P 450 inhibitors, pos. effectors, and alternate substrates for the flavin-contg. **monooxygenase** as well as on studies with the purified hog liver enzyme. MPTP is an excellent substrate for this **monooxygenase** with a Km of 30-33 .mu.M. Limited studies with **human liver** whole homogenates suggest that N-oxygenation is also a major route for the metab. of MPTP in man and the rate of MPTP N-oxide formation is approx. equal to the rate of mitochondrial monoamine oxidase-dependent MPDP+ (1-methyl-4-phenyl-2,3-dihydropyridinium species) prodn.

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E2	26	LOMRI ABDERRAHIM/AU
E3	11 -->	LOMRI N/AU
E4	2	LOMRI NOUR EDDINE/AU
E5	1	LOMRI NOURDINE/AU
E6	14	LOMRI NOUREDINE/AU
E7	6	LOMRI NOUREDINE/AU
E8	2	LOMRI/AU
E9	11	LOMSADZE A V/AU
E10	1	LOMSADZE ALEXANDRE/AU
E11	6	LOMSADZE B/AU
E12	97	LOMSADZE B A/AU
E13	1	LOMSADZE D I/AU
E14	2	LOMSADZE D M/AU
E15	18	LOMSADZE G I/AU
E16	1	LOMSADZE K T/AU
E17	2	LOMSADZE KETEVAN/AU
E18	1	LOMSADZE M SH/AU
E19	1	LOMSADZE N/AU
E20	3	LOMSADZE R/AU
E21	7	LOMSADZE R A/AU
E22	8	LOMSADZE R N/AU
E23	3	LOMSADZE R O/AU
E24	5	LOMSADZE SH YU/AU
E25	2	LOMSADZE T E/AU

09/583,310 Search Strategy/Results

=> S (E3 OR E4 OR E5 OR E6 OR E7) AND (MONOOXYGENASE AND LIVER AND HUMAN)

11 "LOMRI N"/AU  
2 "LOMRI NOUR EDDINE"/AU  
1 "LOMRI NOURDINE"/AU  
14 "LOMRI NOUREDINE"/AU  
6 "LOMRI NOUREDINE"/AU  
10940 MONOOXYGENASE  
456971 LIVER  
972773 HUMAN

L16 5 ("LOMRI N"/AU OR "LOMRI NOUR EDDINE"/AU OR "LOMRI NOURDINE"/AU OR "LOMRI NOUREDINE"/AU OR "LOMRI NOUREDINE"/AU) AND (MONOOXYGENASE AND LIVER AND HUMAN)

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YOU HAVE REQUESTED DATA FROM 5 ANSWERS - CONTINUE? Y/(N):y

L16 ANSWER 1 OF 5 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1996:34513 CAPLUS

DOCUMENT NUMBER: 124:169246

TITLE: Molecular cloning of the flavin-containing monooxygenase (form II) cDNA from adult human liver. [Erratum to document cited in CA118:186740]

AUTHOR(S): Lomri, Nouredine; Gu, Qimin; Cashman, John R.

CORPORATE SOURCE: School Pharm, University California, San Francisco, CA, 94143-0446, USA

SOURCE: Proceedings of the National Academy of Sciences of the United States of America (1995), 92(21), 9910  
CODEN: PNASA6; ISSN: 0027-8424

PUBLISHER: National Academy of Sciences

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The errors were not reflected in the abstr. or the index entries.

L16 ANSWER 2 OF 5 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1995:874565 CAPLUS

TITLE: Molecular cloning of the flavin-containing monooxygenase (form II) cDNA from adult human liver

AUTHOR(S): Lomri, N.; Gu, Q.; Cashman, J. R.

SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1995), 92(21), 9910  
CODEN: PNASA6; ISSN: 0027-8424

DOCUMENT TYPE: Journal; Errata

LANGUAGE: English

AB Unavailable

L16 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1993:664287 CAPLUS

DOCUMENT NUMBER: 119:264287

TITLE: Regio- and stereoselective oxygenations by adult human liver flavin-containing monooxygenase 3. Comparison with forms 1 and 2

AUTHOR(S): Lomri, Nouredine; Yang, Zicheng; Cashman, John R.

CORPORATE SOURCE: Sch. Pharm., Univ. California, San Francisco, CA, 94143-0446, USA

SOURCE: Chem. Res. Toxicol. (1993), 6(6), 800-7  
CODEN: CRTOEC; ISSN: 0893-228X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The cDNA for the adult human liver flavin-contg. monooxygenase (form 3) (FMO3) was cloned, sequenced, and expressed in Escherichia coli. The cDNA-expressed FMO3 was used to investigate the regio- and stereoselective N- and S-oxygenation of a no. of tertiary amines and sulfides, resp. For comparison, the N- and S-oxygenation of the same chems. and drugs were examd. with adult human liver microsomes from a normal healthy female donor and FMO1 from pig liver and FMO2 from rabbit lung. Both cDNA-expressed FMO3 and adult human liver microsomes N-oxygenated trifluoperazine or 10-(N,N-dimethylaminoalkyl)-phenothiazines with similar substrate specificities. The substrate specificity for FMO3 differed, however, from that of pig liver FMO1. Nucleophilic sulfur-contg. compds. [i.e., thiobenzamide, (4-bromophenyl)-1,3-oxathiolane, and 2-methyl-1,3-benzodithiole] were efficiently S-oxygenated by cDNA-expressed FMO3 and adult human liver microsomes. Stereoselective S-oxygenation of (+)- and (-)-(4-bromophenyl)-1,3-oxathiolane and 2-methyl-1,3-benzodithiole was therefore investigated. In general, the stereoselectivity obsd. for

S-oxygenation in the presence of FMO3 was similar to that obsd. in the presence of adult human liver microsomes. In most cases examd., however, the stereoselectivity for S-oxygenation was quite distinct from that obsd. for pig liver FMO1. The authors conclude that FMO3 is the major form of FMO active in adult human liver. Because the stereoselectivity for S-oxygenation and the substrate specificity for tertiary amine N-oxygenation by cDNA-expressed FMO3 are distinct from those of pig liver FMO1, the authors conclude that the binding channel for each isoform is quite different. Like FMO2 from rabbit lung, FMO3 apparently possesses a much smaller substrate binding channel than pig liver FMO1, and this undoubtedly has consequences for tertiary amine and sulfide metab. in humans.

L16 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1993:466327 CAPLUS  
DOCUMENT NUMBER: 119:66327  
TITLE: Expression in Escherichia coli of the flavin-containing monooxygenase D (form II) from adult human liver: Determination of a distinct tertiary amine substrate specificity  
AUTHOR(S): Lomri, Nouredine; Yang, Zicheng; Cashman, John R.  
CORPORATE SOURCE: Sch. Pharm., Univ. California, San Francisco, CA, 94143-0446, USA  
SOURCE: Chem. Res. Toxicol. (1993), 6(4), 425-9  
CODEN: CRTOEC; ISSN: 0893-228X  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB The cDNA for a major component of the family of flavin-contg. monooxygenases (FMOs) present in adult human liver (i.e., HLFMO-D) has been cloned and expressed in a prokaryotic system. Escherichia coli strain NM522 was transformed with pTrcHLFMO-D, and the HLFMO-D cDNA was expressed under the control of the Trc promoter. A variety of tertiary amine substrates [i.e., chlorpromazine and 10-[(N,N-dimethylamino)alkyl]-2-(trifluoromethyl)phenothiazines] were efficiently oxygenated by HLFMO-D cDNA expressed in E. coli or by adult human liver microsomes. Approx. dimensions of the substrate binding channel for both adult human liver microsomal FMO and cDNA-expressed HLFMO-D were apparent from an examn. of the N-oxygenation of a series of 10-[(N,N-dimethylamino)alkyl]-2-(trifluoromethyl)phenothiazines. The substrate regioselectivity studies suggest that adult human liver FMO form D possesses a distinct substrate specificity compared with form A FMO from animal hepatic sources. It is likely that the substrate specificity obsd. for cDNA-expressed adult human liver FMO-D may have consequences for the metab. and distribution of tertiary amines and phosphorus- and sulfur-contg. drugs in humans and may provide insight into the physiol. substrate(s) for adult human liver FMO.

L16 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1993:186740 CAPLUS  
DOCUMENT NUMBER: 118:186740  
TITLE: Molecular cloning of the flavin-containing monooxygenase (form II) cDNA from adult human liver  
AUTHOR(S): Lomri, Nouredine; Gu, Qimin; Cashman, John R.  
CORPORATE SOURCE: Sch. Pharm., Univ. California, San Francisco, CA, 94143-0446, USA  
SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1992), 89(5), 1685-9  
CODEN: PNASA6; ISSN: 0027-8424  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Several cDNA clones encoding the adult human liver flavin-contg. monooxygenase (FMO; dimethylaniline N-oxidase, EC 1.14.13.8) were isolated from .lambda.gt10 and .lambda.gt11 libraries. The cDNA libraries were screened with 3 synthetic 36-mer oligonucleotide probes derived from the nucleic acid sequence of pig liver FMO cDNA. The deduced amino acid sequence for adult human liver FMO was quite distinct from pig liver FMO, and adult human liver FMO was designated form II (HLFMO II). The full-length cDNA sequence of HLFMO II [2119 base pairs (bp)] had an open reading frame of 1599 nucleotides, which encoded a 533-amino acid protein of Mr 59,179, a 5'-noncoding region of 136 nucleotides and a 3'-noncoding region of 369 nucleotides, excluding the poly(A) tail. The deduced amino acid sequence of HLFMO II had 80% similarity with the rabbit



09/583,310 Search Strategy/Results

liver FMO II but only 52%, 55%, and 53% amino acid similarity with rabbit liver (form I), pig liver (form I), and fetal human liver (form I) FMOs, resp. RNA anal. of adult human liver RNA showed that there was one HLFMO II mRNA species. Anal. of genomic DNA indicated that HLFMO II was the product of a single gene. Thus, the deduced amino acid sequence for HLFMO II contained highly conserved residues and suggested that FMO enzymes were closely related and, undoubtedly, derived from the same ancestral gene.

## IUBMB Enzyme Nomenclature

## EC 1.14.13.8

**Common name:** dimethylaniline monooxygenase (*N*-oxide-forming)

**Reaction:** *N,N*-dimethylaniline + NADPH<sub>2</sub> + O<sub>2</sub> = *N,N*-dimethylaniline *N*-oxide + NADP + H<sub>2</sub>O

**Other name(s):** dimethylaniline oxidase; dimethylaniline *N*-oxidase; FAD-containing monooxygenase; *N,N*-dimethylaniline monooxygenase; DMA oxidase; mixed-function amine oxidase; FMO; FMO-I; FMO-II; flavin monooxygenase; flavin-containing monooxygenase

**Systematic name:** *N,N*-dimethylaniline,NADPH<sub>2</sub>:oxygen oxidoreductase (*N*-oxide-forming)

**Comments:** A flavoprotein. Acts on various dialkylarylamines.

**Links to other databases:** [BRENDA](#), [EXPASY](#), [KEGG](#), [WIT](#), CAS registry number: 37256-73-8

**References:**

1. Ziegler, D.M. and Pettit, F.H. Microsomal oxidases. I. The isolation and dialkylarylamine oxygenase activity of pork liver microsomes. *Biochemistry* 5 (1966) 2932-2938. [Medline UI: [67122902](#)]

[EC 1.14.13.8 created 1972]

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Return to [EC 1.14.13 home page](#)

Return to [EC 1.14 home page](#)

Return to [EC 1 home page](#)

Return to [Enzymes home page](#)

Return to [IUBMB Biochemical Nomenclature home page](#)

FMO-III not found

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	29	flavin with monooxygenase	USPAT; EPO; JPO; DERWENT	2002/02/21 15:03
2	L6	13	l1 and liver and human	USPAT; EPO; JPO; DERWENT	2002/02/21 15:03
3	L11	698	monooxygenase	USPAT; EPO; JPO; DERWENT	2002/02/21 15:04
4	L16	167	l11 and liver	USPAT; EPO; JPO; DERWENT	2002/02/21 15:04
5	L21	116	l16 and human	USPAT; EPO; JPO; DERWENT	2002/02/21 15:04
6	L26	103	l21 and type	USPAT; EPO; JPO; DERWENT	2002/02/21 15:06
7	L31	75	l21 and ("3" or III)	USPAT; EPO; JPO; DERWENT	2002/02/21 15:06
8	L36	0	l21 and (type with "3")	USPAT; EPO; JPO; DERWENT	2002/02/21 15:07
9	L41	14	l21 and (type with III)	USPAT; EPO; JPO; DERWENT	2002/02/21 15:07
10	L46	3	fmo3 or fmo-3 or fmo-III or fmoIII	USPAT; EPO; JPO; DERWENT	2002/02/21 15:15